IN THE MATTER OF THE UNITED STATES PATENT APPLICATION SERIAL NO. **10/042,352** IN FAVOUR OF **BERNARD CHARLES SHERMAN**, APPLICANT AND THE INVENTOR OF THE SUBJECT MATTER THEREIN, FILED December 18, 2001.

DECLARATION

I, **Michael Mantle Lipp Ph.D.**, of Alkermes Inc. SOLEMNLY DECLARE AND AFFIRM THE FOLLOWING:

1. I am currently employed at Alkermes Inc. (a pharmaceutical and drug delivery technology company located in Cambridge, Massachusetts) in the position of Director of Formulation Development. Presently, I work in the areas of preformulation, formulation, pilot-scale process development and physicochemical analysis of pharmaceutical compositions for various routes of administration, including injectable, pulmonary and oral. I hold a Ph.D. in Chemical Engineering from the University of California. A copy of my curriculum vitae is attached as **Exhibit 1** to this my Declaration. As such I believe I am well qualified to comment and provide opinion in these matters.

2. The following paragraphs contain my comments and opinions concerning the United States Patent Office Examiner's rejection of claims 1, 6 and 7 in the Final Action, dated March 18, 2004 (hereafter referred to as the Final Action), of U.S. Patent Application No. 10/042,352 entitled "Fosinopril Sodium Tablet Formulation" (hereafter referred to as the '352 patent application).

3. I was asked by Neil H. Hughes, Patent Agent of the firm Ivor M. Hughes Barristers and Solicitors, Patent and Trade Mark Agents and Counsel for the inventor Dr. Bernard Charles Sherman, to provide my opinions concerning the position taken by the United States Patent Office Examiner and the Examiner's rejection of the aforementioned claims of the '352 patent application. In particular, I was asked to provide my opinions with respect to the Examiner's allegation that pending claims 1, 6 and 7 set out in the '352 patent application are unpatentable over Wong (U.S. Patent No. 5,492,904; hereafter referred to as the '904 patent) in view of Sjoerdsma (U.S. Patent No. 4,189,492; hereafter referred to as the '492 patent).

4. As I describe below, in my opinion, the invention described in the claims cited by the Examiner is **not** obvious in light of the combined teachings and disclosures of the '904 and '492 patents. I thus disagree with the conclusions reached by the Examiner in the Final Action with respect to the '352 patent application and these prior art documents. I describe my opinions further below, beginning with a summary of my interpretation of the claimed invention in question of the '352 patent application followed by my opinions with respect to the Examiner's comments and conclusions concerning the teachings and disclosures of the '904 and '492 patents.

Summary of the Invention of the '352 Patent Application

5. The '352 patent application, entitled "Fosinopril Sodium Tablet Formulation" discloses stable tablets comprising fosinopril sodium prepared utilizing either zinc stearate or stearic acid as a lubricant.

6. As described on page 1 of the '352 patent application, U.S. Patent No. 5,006,344 (hereafter referred to as the '344 patent) teaches that tablets containing fosinopril sodium and magnesium stearate as a lubricant display extremely poor stability. The '344 tablet discloses as its invention tablets containing fosinopril sodium with improved stability due to the substitution of either sodium stearyl fumarate or hydrogenated vegetable oil for magnesium stearate as the lubricant, with sodium stearyl fumarate being the preferred lubricant (I note that sodium stearyl fumarate is not a commonly-used lubricant in pharmaceutical practice).

7. As stated on page 1 of the '352 patent application, the object of the invention disclosed therein is to identify an alternative lubricant for fosinopril sodium tablets that is (i) effective as a lubricant, (ii) inexpensive (noting that sodium stearyl fumarate is much more expensive relative to more commonly used tablet lubricants) and (iii) results in the formation of tablets in which fosinopril sodium displays good stability.

8. As described on page 2 of the '352 patent application, it was surprisingly found that the use of either stearic acid or zinc stearate as the lubricant results in the formation of tablets in which fosinopril displays excellent stability in comparison to tablets made utilizing either magnesium or calcium stearate as the lubricant. In particular, it is stated on page 2 of the '352 patent application that:

"It has been found that both stearic acid and zinc stearate are effective lubricants for fosinopril sodium tablets.

Moreover, it has been surprisingly found that, unlike magnesium stearate and calcium stearate, neither stearic acid nor zinc stearate causes the tablets to be unstable.

The invention is thus a pharmaceutical tablet comprising fosinopril sodium along with either stearic acid or zinc stearate as the lubricant."

9. As I will describe further below, the above result is indeed surprising in my opinion, in light of the fact that, considering the four lubricants from the stearic acid family (i.e., stearic acid and three variants of metal ion salts thereof) discussed in the above quote, the use of two of these stearic acid-based lubricants result in tablets containing fosinopril with good stability (i.e., stearic acid and zinc stearate), whereas two of the stearic acid-based lubricants result in tablets containing fosinopril with relatively poor stability (i.e., magnesium and calcium stearate). In my opinion, in light of the significantly poor stability of tablets containing fosinopril sodium and magnesium stearate (noting as well that magnesium stearate is the most commonly-used tablet lubricant in standard pharmaceutical practice), a skilled formulator would not have expected the use of an alternative stearic acid-based lubricant to result in the formation of tablets containing fosinopril sodium with adequate stability. As I will discuss further below, I also feel that this point is evidenced by the teachings and disclosures of the '344 patent; faced with the same issue as that discussed in the '352 patent application (i.e., the instability of fosinopril sodium in tablets made employing

magnesium stearate as a lubricant), the inventors of the '344 patent resorted to the use of two much less commonly-used lubricants outside of the stearic acid family of lubricants.

10. The '352 patent application discloses and teaches on page 2 that, in addition to fosinopril sodium and the lubricant (stearic acid or zinc stearate), the tablets also comprise at least one other excipients as a filler or binder (with lactose being preferred for such use as a filler) and optionally a disintegrant, colouring agent and another active ingredient. The level of lubricant specified as being suitable for use is preferably from about 0.3 to 4.0 percent. The '352 patent application also specifies on page 3 that the tablets of the invention can be prepared via conventional methods such as wet or dry granulation.

11. The '352 patent application contains five illustrative examples, these being the production and accelerated stability testing of five example tablet formulations containing fosinopril sodium (10 mg) and lactose anhydrous (188 mg) in addition to 2 mg of either magnesium stearate (Example 1), zinc stearate (Example 2), calcium stearate (Example 3), stearic acid (Example 4) and sodium stearyl fumarate (Example 5). Accelerated stability testing conditions were 60 °C for a period of two weeks. Samples were analyzed at the end of the two week period via HPLC testing to determine the percent degradation of fosinopril.

12. The stability testing results shown in the table on page 4 of the '352 patent clearly indicate the superiority of the use of zinc stearate as the lubricant versus any of the additional lubricants tested, including either magnesium stearate or calcium stearate, the presence of both of which led to significant

degradation of fosinopril in the tablets tested. In particular, zinc stearate (1.7% degradation), stearic acid (2.1% degradation) and sodium stearyl fumarate (2.8% degradation) all displayed good stability, with the zinc stearate formulation showing the lowest percent degradation. In contrast, the magnesium stearate (46.2% degradation) and calcium stearate (75.5% degradation) formulations displayed extremely poor stability. Thus, in my opinion, the Applicant of the '352 patent application has clearly determined and established by experimentation that the use of zinc stearate as a lubricant in tablets containing fosinopril sodium has the advantageous and unexpected result of minimizing the degradation of fosinopril sodium.

13. In my opinion, the magnitude of the differences in stability in terms of percent degradation for the zinc stearate formulation versus either the magnesium or calcium stearate formulations is indeed a surprising and novel result. Again, in my opinion, a skilled formulator aware of the extent to which fosinopril degrades in the presence of magnesium stearate would not have expected the use of zinc stearate to result in the formulation of tablets containing fosinopril sodium with good stability.

The Examiner's Assertions Concerning the Obviousness of the Claims of the '352 Patent Application in Light of the Teachings and Disclosures of Wong and Sjoerdsma

14. In the Final Action, the Examiner states that claims 1, 6 and 7 of the '352 patent application are unpatentable over the '904 patent (Wong) in view of the '492 patent (Sjoerdsma). With respect to the teachings and disclosures of the

'904 patent, the Examiner states that this patent discloses a tablet comprising fosinopril sodium, lactose and stearic acid. For example, it is stated on page 2 of the final action that:

> *"Wong et al. discloses a tablet comprising fosinopril sodium, lactose and stearic acid (col. 4, line. 30-58). This reference however does not disclose zinc stearate as the lubricant."*

15. With respect to the teachings of the '492 patent, the Examiner states that this patent discloses anti-hypertensive tablet formulations for which zinc stearate is utilized as a lubricant. For example, it is stated on page 2 of the Final Action that:

> *"Sjoerdsma discloses an anti-hypertensive tablet formulation where zinc stearate is used to lubricate the formulation (col. 4, lin. 28-30). It would be obvious to a skilled artisan to use the zinc stearate in order to lubricate the granules of the tablet formulation and improve stability."*

16. The Examiner alleges that the combined teachings of the '904 and '492 patents makes obvious the claims in question of the '352 patent application. In particular, the Examiner alleges that a skilled formulator would have substituted zinc stearate as taught by the '492 patent (Sjoerdsma) for use as the lubricant in the formulations disclosed in the '904 patent (Wong) in order to improve stability and optimize the delivery of fosinopril sodium. For example, it is stated on page 3 of the Final Action that:

"With this in mind, it would have been obvious to one of ordinary skill in the art to substitute the lubricant of Sjoerdsma in order to lubricate the tablet formulation and improve stability. A skilled artisan would have been motivated to modify the formulation presented in order to optimize the delivery of the active agent. It would have been obvious to combine and optimize the formulations of the prior art with the expected result of an anti-hypertensive tablet formulation with improved stability."

17. The Examiner also alleges that there would be sufficient motivation for a skilled formulator to combine the teachings of the '904 and '492 patents in part in order to improve the stability of as well as to properly lubricate the formulations containing fosinopril sodium purportedly taught by the '904 patent. For example, it is stated on pages 4 and 5 of the Final Action that:

"Since Wong provides the basic formulation, save the specific lubricant of newly amended claims, and Sjoerdsma provides a similar tablet formulation comprising an active agent, lactose and a lubricant (zinc stearate), the motivation would be to improve the stability of the formulation and properly lubricate the tableting formulation. It remains the position of the examiner that barring a showing of criticality to the specific lubricant over those of the prior art combination at a particular concentration where an unexpected result is reached, the claims will remain obviated by the prior art."

18. As I describe below, I disagree with the Examiner with respect to the Examiner's allegations concerning the combined teachings and disclosures of the '904 and '492 patents. In my opinion, no combinations of the teachings and

disclosures of these two patents make obvious the invention of the '352 patent application. I first provide my interpretation of the '904 and '492 patents below, followed by my opinions concerning the combined teachings of the '904 and '492 patents and their relevance or lack thereof to the '352 patent application.

Teachings and Disclosures of the '904 Patent (Wong)

19. The '904 patent, entitled "Composition of Angiotensin-II Receptor Antagonists and Calcium Channel Blockers", discloses combination formulations comprised of an angiotensin-II receptor antagonist in combination with a calcium channel blocker for use in the treatment of hypertension and congestive heart failure. For example, it is stated in column 1 of the '904 patent that (lines 16 through 21):

> *"This invention relates to novel pharmaceutical compositions containing an angiotensin-II receptor antagonist from a selected class in combination with a calcium channel blocker from a selected class useful for the treatment of hypertension and for the treatment of congestive heart failure."*

20. The inventor of the '904 patent also teaches the potential for inclusion of a wide range of additional therapeutic agents for the treatment of hypertension in the disclosed combination angiotensin-II receptor antagonist:calcium channel blocker formulations. For example, it is stated in column 4 of the '904 patent that (lines 13 through 16):

"The combinations of compounds can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination with additional therapeutic agents."

Following this quote in lines 16 through 36 of the '904 patent is an extensive list of over 50 additional antihypertensives, diuretics and angiotensin converting enzyme (ACE) inhibitors specified as being suitable for inclusion in the disclosed formulations; fosinopril sodium is listed in line 34 of the '904 patent along with additional ACE inhibitors such as enalapril, captopril and quinapril.

21. With respect to pharmaceutical dosage forms, the inventor of the '904 patent teaches that a wide range of dosage forms are suitable for practicing the invention disclosed therein, including tablets, capsules, solutions for injection, etc. For example, it is stated in column 4 of the '904 patent that (lines 48 through 52):

"The active ingredients can be administered orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixir syrups, and suspensions. They can also be administered parenterally, in sterile liquid dosage forms.

22. With respect to tablet and capsule dosage forms, the '904 patent provides only limited and generic information concerning the excipients suitable for use; magnesium stearate and stearic acid are included among the excipients listed. For example, it is stated in column 4 of the '904 patent that (lines 53 through 63):

*"**Gelatin capsules contain the active ingredients and powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets.** Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract."* (Emphasis added.)

23. The '904 patent also provides generic information illustrating the production of tablets in column 6, lines 1 through 8; said tablets are described to be comprised of unspecified active ingredients in conjunction with colloidal silicon dioxide, magnesium stearate, microcrystalline cellulose, starch and lactose. The specific method of manufacture of said tablets is also left unspecified (lines 3 and 4 from column 6 of the '904 patent teach the use of "conventional procedures").

24. The '904 patent contains a single claim, that being a method of treating hypertension comprised of the administration of a therapeutically synergistic combination of a specific angiotensin-II receptor antagonist (listed in claim 1 of the '904 patent) with diltiazem as a calcium channel blocker.

25. As I will describe in more detail below with respect to my opinions concerning the combined teachings of the '904 and '492 patents, the '904 patent, read alone or together with the '492 patent, does not in my opinion teach,

disclose or make obvious the invention of the '352 patent application. In my opinion, in addition to the fact that the '904 patent contains no teachings whatsoever concerning stable tablets comprised of fosinopril sodium and zinc stearate, it will be important to consider the following concerning the teachings and disclosures the '904 patent alone when evaluating the combined teachings of the '904 and '492 patents:

i. The '904 patent focuses on pharmaceutical compositions comprised of a combination of an angiotensin-II receptor antagonist and a calcium channel blocker; fosinopril sodium is mentioned only once in the '904 patent as part of a list of over 50 third drug compounds that could potentially be included in the disclosed combination compositions.

ii. As indicated by i., the '904 patent provides no information whatsoever concerning the stability of fosinopril sodium in any pharmaceutical composition.

iii. The only information concerning stability in general contained in the '904 patent is a mention of stabilizers such as antioxidants for inclusion in parenteral solutions in column 5 of the '904 patent.

iv. The '904 patent contains only limited and generic information concerning the production of the disclosed combination pharmaceutical compositions as tablet or capsule dosage forms.

v. As I will describe further below, the specification of the suitability for use of magnesium stearate as a formulation lubricant indicates that the inventor of the '904 patent had no understanding concerning the nature of the problem solved by the invention of the '352 patent application, namely

the use of zinc stearate as a lubricant to provide for the production of stable tablets containing fosinopril sodium.

Teachings and Disclosures of the '492 Patent (Sjoerdsma)

26. The '492 patent, entitled "Antihypertensive Compositions of 2-[1-(2,6-Dichlorophenoxy)Ethyl]-4,5-Dihydro-1H-Imidazole and N-(2-Chloroethyl)-N-(1-Methyl-2-Phenoxy-Ethyl)Benzenemethanamine", discloses combination compositions containing lofexidine (described to be a potent centrally-acting antihypertensive agent in the '492 patent, which is no longer the case for this drug as I will describe further below) and phenoxybenzamine. For example, it is stated in column 2 of the '492 patent that (lines 3 through 10):

> *"This invention relates to novel therapeutic compositions of lofexidine and phenoxybenzamine. More particularly, this invention relates to antihypertensive compositions comprising dosage units administered daily comprising from 0.1 to 1.0 milligrams of lofexidine and from 0.5 to 15 milligrams of phenoxybenzamine, or each of their pharmaceutically acceptable salts, in combination with an inert pharmaceutical carrier."*

27. The novelty and focus of the invention of the '492 patent is described by the inventor to be the surprising result that the co-administration of phenoxybenzamine with lofexidine purportedly enhances the antihypertensive effect of lofexidine (noting again that, as I will describe further below, lofexidine has subsequently been demonstrated to lack efficacy as an antihypertensive

agent and is no longer sold for such purpose). For example, it is stated in column 1 of the '492 patent that (lines 56 through 60):

"I have discovered that the conjoint administration of certain combinations of phenoxybenzamine and lofexidine actually enhances the antihypertensive effect obtained with lofexidine alone, thereby permitting the overall administration of decreased amounts of lofexidine to patients."

28. The inventor of the '492 patent teaches in column 3, lines 26 through 44 of the '492 patent that a wide variety of different unit dosage forms may be utilized in practicing the invention disclosed therein, including tablets, capsules, lozenges, elixirs, emulsions, clear liquid solutions, suspensions, intravenous or intradermal preparations, etc.

29. With respect to the nature and types of excipients suitable for practicing the invention disclosed therein, it is stated in column 3 of the '492 patent that (lines 44 through 66):

"The combination of lofexidine and phenoxybenzamine is most advantageously administered as a pharmaceutical composition in conjunction or admixture with additional organic or inorganic pharmaceutical excipients. ***Suitable solid excipients include inert diluents, for example, calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, as for example, maize starch or alginic acid; binding agents, as for example various starches, gelatin, lactose or acacia mucilage, and lubricating agents, such as***

magnesium stearate, stearic acid or talc. *Suitable liquid excipients include water and alcohols, such as ethanol, benzyl alcohol and the polyethylene alcohols, either with or without the addition of a surfactant. In general, the preferred liquid excipients, particularly for injectable preparations include water, saline solution, dextrose and glycol solutions such as aqueous propylene glycol or an aqueous solution of polyethylele glycol. Liquid preparations to be used as sterile injectable solutions will ordinarily contain from about 0.1% to about 25% by weight and preferably from about 0.1% to about 10% by weight of the active ingredients in the solution."* (Emphasis added.)

Thus, with respect to solid dosage forms, the '492 provides generic information describing the use of conventional tablet and capsule excipients such as diluents, disintegrating agents, lubricants and the like. With specific respect to lubricants, as described in the quote above, magnesium stearate, stearic acid and talc are provided as representatives of this class of excipients.

30. As noted by the Examiner, zinc stearate is mentioned in the '492 patent in the context of a description concerning the formulation of tablets containing lofexidine and phenoxybenzamine via wet granulation. For example, it is stated in column 4 of the '492 patent that (lines 13 through 30):

"Formulations for oral use may be presented as hard or soft shell gelatin capsules containing only the active ingredients, or containing the active ingredients in admixture with a solid diluent, as for example lactose, sorbitol, calcium carbonate, calcium phosphate or kaolin. Tablets containing lofexidine and phenoxybenzamine can be prepared by the conventional wet granulation

method which consists of moistening the dry powders, with or without the addition of an adhesive substance, until the whole is converted into a crumbly mass. Well-known moistening agents such as water or other solvents can be employed. In addition, it is a common practice to add a substance such as gelatin, starch or gum acacia in order to assist in granulating these materials. ***The granules so prepared can be lubricated by dusting or dry blending with a lubricant such as talc or zinc stearate and compressed in the usual manner known in the art.***" (Emphasis added.)

31. Three of the five examples provided in the '492 patent concern the preparation of solid dosage forms containing lofexidine and phenoxybenzamine, these being Example 1 (tablets), 2 (capsules) and 4 (layered slow/rapid-releasing tablets). Talc and calcium stearate are specified as formulation lubricants for the tablets of Example 1 and the capsules of Example 2; zinc stearate is specified as the lubricant for preparation of the slow-release layer from Example 4.

32. The '492 patent contains 6 claims, the first two of which are composition claims (Claim 1 claims a composition containing lofexidine and phenoxybenzamine or salts thereof in combination with an unspecified pharmaceutically acceptable carrier; Claim 2 claims the composition of Claim 1 in the form of a tablet), and the last four of which are method claims.

33. As I will discuss in more detail below with respect to my opinions concerning the combined teachings of the '904 and '492 patents, in my opinion, the '492 patent, read alone or together with the '904 patent, does not teach, disclose or make obvious the invention of the '352 patent application. In my

opinion, in addition to the fact that the '492 patent contains no teachings concerning stable tablets comprised of fosinopril sodium and zinc stearate, it will be important to consider the following concerning the teachings and disclosures the '492 patent alone when evaluating the combined teachings of the '904 and '492 patents:

i. The '492 patent focuses on pharmaceutical compositions comprised of a combination of lofexidine (described to be a potent antihypertensive agent at the time of filing of the '492 patent, which was no longer the case as of approximately 1990) and phenoxybenzamine; fosinopril sodium is not mentioned anywhere in the '492 patent.

ii. The '492 patent provides no information whatsoever concerning the stability of fosinopril sodium in any pharmaceutical composition.

iii. The '492 patent provides no information whatsoever concerning the stability of any pharmaceutical composition or the use of stabilizers, etc.

iv. The '492 patent contains only limited and generic teachings concerning the production of the disclosed combination pharmaceutical compositions as tablet or capsule dosage forms; stability in general or methods and practices to ensure formulation stability are not part of these teachings.

v. As I will describe further below, lofexidine (i) possesses a chemical structure very different than that of fosinopril and (ii) was known to be ineffective as an antihypertensive agent at the time of filing of the '904 patent.

vi. As I will also describe further below, the specification of the suitability for use of magnesium and calcium stearate as lubricants indicates that the inventor of the '492 patent had no understanding concerning the nature of

the problem solved by the invention of the '352 patent application, namely the use of zinc stearate as a lubricant to provide for the production of stable tablets containing fosinopril sodium (as I indicated in paragraph 12 above, the use of calcium stearate as the tablet lubricant resulted in the largest amount of degradation seen among the tablets tested as described in Examples 1 through 5 of the '352 patent application).

Combined Teachings of the '904 (Wong) and '492 (Sjoerdsma) Patents:

34. As I described above, the Examiner alleges that the combined teachings of the '904 and '492 patents makes obvious the claims in question of the '352 patent application. In particular, the Examiner alleges that a skilled formulator would have utilized zinc stearate as taught by the '492 patent (Sjoerdsma) as the lubricant in the formulations disclosed in the '904 patent (Wong) in order to improve stability and optimize the delivery of fosinopril sodium. As is shown above in paragraph 16, the Examiner states on page 3 of the Final Action that:

"With this in mind, it would have been obvious to one of ordinary skill in the art to substitute the lubricant of Sjoerdsma in order to lubricate the tablet formulation and improve stability. A skilled artisan would have been motivated to modify the formulation presented in order to optimize the delivery of the active agent. It would have been obvious to combine and optimize the formulations of the prior art with the expected result of an anti-hypertensive tablet formulation with improved stability."

35. The Examiner further states on pages 4 and 5 of the Final Action that:

"Regarding argument b., it is the position of the examiner that the combination provides sufficient motivation. Wong discloses a tablet formulation comprising fosinopril sodium along with a lubricant. The fact that the active agent is secondary is irrelevant since the opened claims language does not exclude further active agents. Furthermore the inclusion of lactose and other lubricants are very well known in the art. Barring a showing of criticality to eh (SIC) *inclusion of zinc stearate over any other lubricant, would be required to establish the patentability of the formulation. Since Wong provides the basic formulation, save the specific lubricant of newly amended claims, and Sjoerdsma provides a similar tablet formulation comprising an active agent, lactose and a lubricant (zinc stearate), the motivation would be to improve the stability of the formulation and properly lubricate the tableting formulation. It remains the position of the examiner that barring a showing of criticality to the specific lubricant over those of the prior art combination at a particular concentration where an unexpected result is reached, the claims will remain obviated by the prior art."*

36. I disagree with the Examiner with respect to the Examiner's interpretation of the combined teachings of the '904 and '492 patents as described above for several reasons. In my opinion, it would <u>not</u> have been obvious to combine and optimize the formulations of the '904 and '492 patents in the manner that the Examiner has described (i.e., utilizing the teaching of the use of zinc stearate in the '492 patent in practicing the tablet formulations containing fosinopril sodium, lactose and a lubricant taught in the '904 patent).

37. First, as I described above, neither the '904 patent nor the '492 patent discuss the stability of any drug, including fosinopril sodium, in a tablet or capsule formulation. These patents do not provide any teachings concerning methods to achieve the stabilization of any antihypertensive, including fosinopril sodium, in any solid dosage form. I thus do not believe that a skilled formulator would be motivated to combine the teachings of the '904 and '492 patents with the goal of achieving a stabilized tablet formulation containing fosinopril sodium, lactose and zinc stearate, in addition to other excipients.

38. Second, as evidenced by the comments made by the Examiner in the quotes shown above, the Examiner seems to imply that a skilled formulator would believe that a given method of stabilization of a dosage form containing a specific antihypertensive agent would apply to all antihypertensive agents. In my opinion, a skilled formulator would understand that this was far from the case.

39. The drug class of antihypertensive agents encompasses a wide range of chemically diverse and structurally distinct molecules. As illustrative of this, I have included Chapter 33 entitled "Antihypertensive Agents and the Drug Therapy of Hypertension" from the standard textbook entitled "Goodman and Gilman's - The Pharmacological Basis of Therapeutics" (Eighth Edition, 1990) as **Exhibit 2** to this my Declaration. As shown in Table 33-1 from this reference, as of 1990, there were known to be five major classes of antihypertensive agents, these being: (A) diuretics, (B) sympatholytic drugs, (C) vasodilators, (D) calcium channel blockers and (E) angiotensin converting-enzyme (ACE) inhibitors (I note

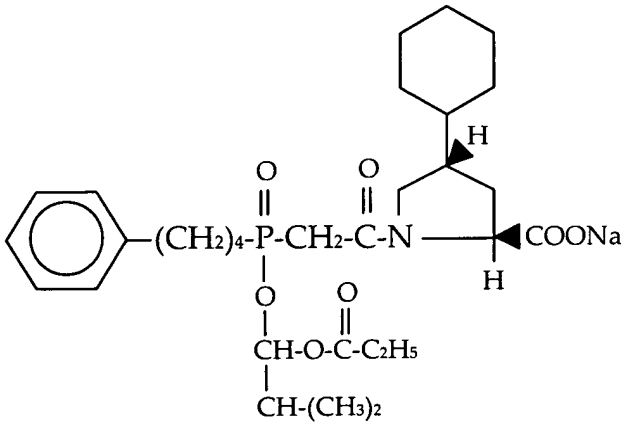
that the Eight Edition of this reference was published in 1990; as is indicated by the '904 patent, class E has since expanded to include angiotensin-II receptor antagonists in addition to ACE inhibitors). The drug molecules from among these five classes exhibit significant structural and physicochemical diversity, ranging from thiazides to beta-adrenergic agonists to carboxyalkyldipeptide derivatives, etc. As I discuss further below, in my opinion, a skilled formulator would in no way expect a common method of stabilization to apply to all such antihypertensive drugs.

40. In support of this, it is well known that even within a given class of antihypertensive agents, the stability properties (mechanisms of degradation, mechanisms of stabilization via various excipients, etc.) of the antihypertensive drugs within the class can vary widely. An example of this is the class of ACE inhibitors (Class E from Table 33-1 of **Exhibit 2**) of which fosinopril sodium is a member.

41. ACE inhibitors are a diverse class of antihypertensive drugs that are structurally based on a variety of dipeptide derivatives, namely N-carboxyalkyldipeptide derivatives. As described in the article entitled "The Quantitative Determination of Several Inhibitors of the Angiotensin-Converting Enzyme by CE" from the Journal of Pharmaceutical and Biomedical Analysis (Volume 25 (2001), pp. 775-783) included here as **Exhibit 3** to this my Declaration, ACE inhibitors can be grouped into four subclasses based on their structure types, these being (1) thiol-based (captopril, etc.), (2) second carboxyl group-containing (enalaprilat, lisinopril), (3) carboxylic acid ethyl ester-

containing (prodrugs; enalapril, quinapril, etc.) and (4) phosphorous-containing (prodrugs; fosinopril).

42. Fosinopril sodium, designated as [1[S*(R*)]2α,4β]-4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)-propoxy](4-phenylbutyl)phosphinyl]-acetyl]-L-proline, sodium salt, is the primary representative of the phosphorous-containing ACE inhibitor subclass. The chemical structures of enalapril, lisinopril, quinapril and fosinopril are shown in Figure 1 from **Exhibit 3**; I have shown the chemical structure of fosinopril sodium below:



43. The mechanisms of degradation and the stability in the presence of various excipients of members of the different subclasses of ACE inhibitors are known to vary significantly. For example, as discussed in Section D entitled "ACE Inhibitors" of Chapter 20 from the reference entitled "Solid-State Chemistry of Drugs published in 1999 included here as **Exhibit 4** to this my Declaration, the N-carboxyalkyldipeptide carboxylic acid ethyl ester-containing (i.e. prodrug) subclass of ACE inhibitors that includes enalapril, moexipril and

quinapril are well-known to degrade via hydrolysis and/or cyclization (diketopiperazine formation).

44. There are several examples in the literature of the stabilization of drug molecules from this subclass of ACE inhibitors via the use of alkaline stabilizers, including alkaline magnesium compounds. One of these is U.S. patent 4,743,450 (hereafter referred to as the '450 patent) that I have included here as **Exhibit 5** to this my Declaration discloses and claims formulations and processes for the creation of stable dosage forms containing ACE inhibitors such as enalapril and quinapril via the use of alkaline magnesium compounds as stabilizers. For example, it is stated in column 1 of the '450 patent that (lines 1 through 22):

"STABILIZED COMPOSITIONS

BACKGROUND

Certain ACE (Angiotensin Converting Enzyme) inhibitors, which are useful as antihypertensives, are susceptible to certain types of degradation. Specifically, quinapril and structurally-related drugs can degrade via (1) cyclization via internal nucleophilic attack to form substituted diketopiperazines, (2) hydrolysis of the side chain ester group, and (3) oxidation to form products having often unwanted coloration.

THE INVENTION

It has been discovered that stable compositions containing ACE inhibitors of the type discussed above can be produced using certain additives as stabilizers.

In one embodiment, 8.6 wt % magnesium carbonate is combined with 5.4 wt % quinapril hydrochloride with the inclusion of 38.0 wt % lactose to yield a composition which withstands oxidative, hydrolytic, and cyclization degradation at 60° C. for one month."

45. In contrast, for the case of fosinopril sodium, it has been demonstrated that (i) fosinopril sodium degrades via a different mechanism than that discussed above for enalapril, quinapril and moexipril and (ii) this degradation is caused by several metal ions that include magnesium. For example, the 1993 article from the journal Pharmaceutical Research entitled "Mechanism and Kinetics of Metal Ion-Mediated Degradation of Fosinopril Sodium" that I have included here as **Exhibit 6** to this my Declaration discusses the results of studies examining the mechanism of degradation of fosinopril sodium in the presence of several metal ions and its relevance to the stability of fosinopril sodium in solid dosage forms. With respect to the nature of these studies, it is stated on page 800 of **Exhibit 6** that:

"In this communication we report a novel metal ion mediated rearrangement that results in degradation of fosinopril into a β-ketoamide, III, and a phosphonic acid, IV. The degradation product III was isolated from the tablets undergoing accelerated stability testing and was characterized by 1H NMR and MS. Its structure was confirmed by unambiguous synthesis. Compound IV is reported in the literature (2). We show that the degradation/rearrangement of fosinopril is caused by several metal ions, in particular magnesium. A mechanism involving

metal chelation is proposed for the degradation of fosinopril sodium by this process."

46. Scheme I from page 801 of **Exhibit 6** depicts the identified mechanisms and pathways for degradation of fosinopril sodium; these metal ion-mediated mechanisms and pathways are distinct and different from those related to the cyclization of such ACE inhibitors as enalapril and quinapril as described above (noting that fosinopril sodium also undergoes hydrolysis to the active form fosinoprilat).

47. Additionally, in support of the information disclosed in the '344 patent with respect to the instability of fosinopril sodium in the presence of magnesium stearate that I discussed in paragraph 6 above, **Exhibit 6** also specifically teaches the degradation of fosinopril sodium in the presence of magnesium stearate. For example, it is stated on page 802 of **Exhibit 6** that:

"Also shown in Fig. 1 (A and C) are the chromatograms of an equimolar reaction mixture of fosinopril and magnesium stearate after similar treatment in methanol. From the products formed, it was confirmed that both magnesium stearate and magnesium acetate reacted with fosinopril in a similar manner. Other metal ion acetates exhibited similar reactivity towards fosinopril."

48. The authors of **Exhibit 6** further teach that the solution stability results described therein are also relevant to solid dosage forms such as tablets containing fosinopril sodium in combination with lubricants such as magnesium stearate. For example, it is stated on page 809 of **Exhibit 6** that:

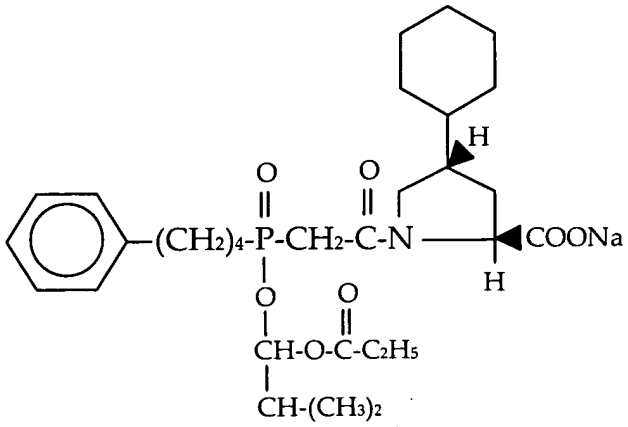
"Significance to Tablet Formation

The results of this study helped rationalize the degradation of fosinopril in the tablet formulation lubricated with magnesium stearate. It clearly identified two distinct pathways of degradation, i.e., magnesium ion mediated and hydrolysis. In the tablet formulation the amount of the lubricant is low compared to drug and hence the magnesium ion-mediated degradation would occur only to a small extent as predicted by the second order kinetic model. However, the formation of acidic degradation products would enhance the acid catalyzed degradation of the ester prodrug. In Fig. 7 stability data from an experimental fosinopril tablet formulation containing magnesium stearate as lubricant and stored at 50°C and 100% relative humidity are shown. The formation of magnesium ion-mediated product III levels off, whereas the formation of hydrolysis product II continues with time of storage. The data thus validate the predictions of the model." (Emphasis added.)

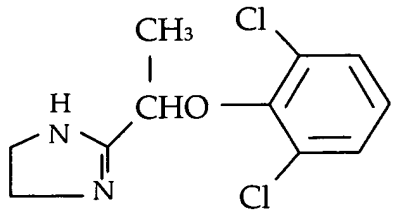
49. Thus, whereas **Exhibit 5** (the '450 patent) teaches the stabilization of the ACE inhibitors quinapril and enalapril in the presence of an alkaline magnesium compound, **Exhibit 6** teaches the instability of the ACE inhibitor fosinopril sodium in the presence of several magnesium-containing compounds, including magnesium stearate. As I described above, such results indicating significant differences in the stability properties (mechanisms of degradation, mechanisms of stabilization via various excipients, etc.) among members of the same class of antihypertensive drugs (i.e., ACE inhibitors) would **not** indicate to

a skilled formulator that that a given method of stabilization of a dosage form containing a specific antihypertensive agent would apply to all antihypertensive agents. As a result, I again do **not** see the motivation that a skilled formulator would have to combine the teachings of the '904 and '492 patents in the manner alleged by the Examiner as described above.

50. In further support of this point, I have included the entries for fosinopril sodium and lofexidine from the standard pharmaceutical reference entitled "The Merck Index" (Eleventh Edition, 1989) as **Exhibit 7** to this my Declaration. As I described in paragraph 42 above, fosinopril sodium, designated chemically as [1[S*(R*)]2α,4β]-4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)-propoxy](4-phenylbutyl)phosphinyl]-acetyl]-L-proline, possesses the structure shown below:



51. In contrast, the structure of lofexidine, structurally related to clonidine which is a member of class B (sympatholytic drugs) shown in Table 33-1 from **Exhibit 7** and designated chemically as 2-[1-(2,6-dichlorophenoxy)ethyl]-4,5-dihydro-1H-imidazole, is shown below:



As is clearly evidenced by their differing structures, functional groups and molecular weights, in my opinion, a skilled formulator would have **no** expectation that there would be any relation between the stabilities of these two different drugs in a given pharmaceutical composition (again noting that the '492 patent contains no teachings with respect to the stability of lofexidine in any solid or solution-based dosage form to begin with).

52. With final respect to this point, I note that, well prior to the filing date of the '904 patent (July 28, 1994), it was known as part of the state of the art that lofexidine was ineffective as an antihypertensive agent and was thus removed from the market for such use. In support of this, it is stated on page 1385 of the article entitled "Lofexidine and Opioid Withdrawal" from the Lancet (Vol. 345, June 3, 1995, pp. 1385-1386) that I have included here as **Exhibit 8** to this my Declaration that:

"Lofexidine (BritLofex, Britannia Pharmaceuticals) is a structural analogue of the antihypertensive agent clonidine.[1] Lofexidine was originally licensed as an anti-hypertensive in Germany, but was withdrawn because of lack of clinical efficacy.

Lofexidine was relaunched in October, 1992, in the UK and is now licensed and promoted as an aid to opioid detoxification."

As a result, a skilled formulator considering the teachings of the '904 patent would not in my opinion have considered lofexidine as an antihypertensive agent. Thus, in my opinion, a skilled formulator would have had no motivation to combine the teachings of these patents based solely on the fact that they both dealt with formulations of antihypertensive agents.

53. As a result, I disagree with the Examiner with respect to the Examiner's allegation that a skilled formulator would have utilized zinc stearate as taught by the '492 patent (Sjoerdsma) as the lubricant in the formulations disclosed in the '904 patent (Wong) in order to improve stability and optimize the delivery of fosinopril sodium. Again, I do **not** see any motivation for a skilled formulator to combine the teachings of the '904 and '492 patents in the manner alleged by the Examiner. In my opinion, neither the '904 patent nor the '492 patent, nor the combination thereof teach any information concerning the nature of the problem solved by the invention of the '352 patent application, namely the use of zinc stearate as a lubricant to provide for the production of tablets containing fosinopril sodium with superior stability. Further, the disclosures of the '904 and '492 patents give no indication that the inventors thereof had any understanding of the nature of the problem solved by the invention of the '352 patent application.

54. Finally, in my opinion, additional teachings contained in **Exhibit 6** (the 1993 article from the journal Pharmaceutical Research entitled "Mechanism

and Kinetics of Metal Ion-Mediated Degradation of Fosinopril Sodium") are worth noting with respect to the lack of obviousness of the invention of the '352 patent application. As I described above in paragraphs 45 through 49 of this my Declaration, **Exhibit 6** teaches that (i) as demonstrated by solution-phase stability studies, several metal ions that include magnesium catalyze and accelerate the degradation of fosinopril sodium and (ii) the results of such studies are relevant to solid dosage forms containing fosinopril sodium in addition to metal ion-containing excipients such as magnesium stearate.

55. In addition to magnesium, **Exhibit 6** specifically teaches that calcium and zinc also cause such degradation when combined with fosinopril sodium. For example, it is stated on pages 804 and 805 of **Exhibit 6** that:

> *"In extended stability studies of the bulk drug substance, fosinopril sodium does not undergo the postulated rearrangement and degradation reactions. If exposed to high humidity, the ester prodrug undergoes hydrolysis to form the active moiety II. In the formulations containing magnesium stearate, fosinopril degrades to form not only II, but also small amounts of III and IV. We have studied the degradation of fosinopril in a model system wherein the fosinopril was reacted with a soluble salt form of a metal in methanol.* ***The solid-state behavior of fosinopril in tablet formulation was simulated in solution by substituting freely soluble magnesium acetate for magnesium stearate. We have demonstrated that the reaction was not unique to magnesium ions but occurred with other ions as well."***

56. Table I. from **Exhibit 6** entitled "Relative Reactivities of Metal Ions in the Degradation of Fosinopril in Methanol at 24° C." shows stability data for the case of 10 different metal ions, including magnesium, calcium and zinc; as evidenced by the data shown in Table I., significant degradation of fosinopril is observed for all of these ions, including zinc. As a result, in my opinion, a skilled formulator aware of these results and knowing the poor stability of tablets containing fosinopril sodium and magnesium stearate would not have expected the use of zinc stearate to result in the formulation of tablets containing fosinopril sodium with good stability. Again, in my opinion and as I described in paragraph 13 above, **Exhibit 6** further indicates that the magnitude of the differences in stability observed as described in the '352 patent application for the zinc stearate formulation versus either the magnesium or calcium stearate formulations is a surprising and nonobvious result.

57. In summary, it is my opinion that the invention disclosed in the '352 patent application are **not** made obvious by any combination of the teachings and disclosures of the '904 (Wong) and '492 (Sjoerdsma) patents. I thus disagree with the Examiner with respect to the Examiner's allegations concerning the combined teachings and disclosures of these two patents.

58. I solemnly declare and affirm further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of

the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereof.

Michael Mantle Lipp

Director, Formulation Development

Alkermes Inc.

AFFIRMED before me)
at Middlesex Cty)
in Cambridge MA, U.S.A.)
this 9th day of February, 2005)

A Commissioner, Notary Public
for taking Oaths

CINDY A. SRAGG
Notary Public
My Commission Expires
January 23, 2009

This is Exhibit 1 referred to in the Declaration of Michael M. Lipp, sworn this 9th day of February, 2005.

A Notary Public

CINDY A. SRAGG
Notary Public
My Commission Expires
January 23, 2009

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  > Coordinate formulation development and analysis efforts for Alkermes partnered and proprietary pulmonary project teams for both pulmonary and injectable formulations
  > Direct pilot process development group developing unit operations and processes for the production of Alkermes formulations
  > Budget, plan and execute internal and external formulations research and feasibility project efforts
* **Direct Alkermes Technology Development Program**
  > Budget, plan and execute internal and external technology development efforts
  > Initiate and facilitate collaborative efforts with external academic and industrial laboratories
  > Perform competitive intelligence and assess novel technologies for acquisition and development
* **Intellectual Property Technical Coordinator**
  > Interface with Alkermes Inc. Intellectual Property Department and AIR Project Teams with respect to AIR intellectual property issues

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  > Coordinate formulation development and analysis efforts for Alkermes partnered and proprietary pulmonary project teams
  > Budget, plan and execute internal and external pulmonary research and feasibility project efforts
* **Direct Technology Development Department**
  > Budget, plan and execute internal and external technology development efforts
  > Initiate and facilitate collaborative efforts with external academic and industrial laboratories
  > Perform competitive intelligence and assess novel technologies for acquisition and development
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Microscopy Society of America Presidential Student Award Winner, 1996
University of California Regents Special Fellowship, 1992-1996

Selected Publications:

1. *Phase and Morphology Changes in Lipid Monolayers Induced by SP-B Protein and its Amino-Terminal Peptide*, M. Lipp, K. Lee, J. Zasadzinski, A. Waring, Science, 273: 1196-1199 (1996).
2. *Solving Medical Problems with Chemical Engineering*, M. Lipp, K. Lee, J. Zasadzinski, A. Waring, Chemtech, 3: 42-57 (1997).
3 *Fluorescence, Polarized Fluorescence, and Brewster Angle Microscopy of Palmitic Acid and Lung Surfactant Protein B Monolayers*, M. Lipp, K. Lee, J. Zasadzinski, A Waring, Biophysical Journal, 72:2783-2804 (1997).
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8. *Production and Characterization of Large Porous Particles for Pulmonary Drug Delivery*, R. Batycky, J. Nice, D. Chen, J. Sung, M. Lipp, J. Mintzes, C. Dunbar, R. Niven, D. Edwards, Materials Research Society Symposium Proceedings, Volume 550 (1999).
9. *Fractal-Dimension Particles for Drug Delivery*, D. Edwards, J. Wright, M. Lipp, R. Batycky, Abstracts of Papers of the American Chemical Society, 219:81-Coll., Pt. 1 March 26 (2000).
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12. *Effects of Lung Surfactant Proteins, SP-B and SP-C, and Palmitic Acid on Monolayer Stability*, J. Ding, D. Takamoto, A. von Nahmen, M. Lipp, K. Lee, A. Waring, J. Zasadzinski, Biophysical Journal, 80 (5): 2262-2272 (2001).
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14. *Interaction of Lung Surfactant Proteins with Anionic Phospholipids,* D. Takamoto, M. Lipp, A.von Nahmen, K. Lee, A. Waring, J. Zasadzinski, Biophysical Journal 81 (1), 153-169 (2001).
15. *Biocompatibility of Lipid-Protein-Sugar Particles Containing Bupivacaine in the Epineurium,* D. Kohane, M. Lipp, R. Kinney, D. Anthony, D. Louis, N. Lotan, R. Langer, Journal of Biomedical Materials Research, 59 (3): 450-459 (2002).

Selected Patents and Published Patent Applications:

1. *Use of Simple Amino Acids to Form Porous Particles*, R. Batycky, M. Lipp, R. Niven, U.S. Patent No. 6,586,008 (July 1, 2003).
2. *Modulation of Release from Dry Powder Formulations*, S. Basu, J. Hrkach, G. Caponetti, M. Lipp, K. Elbert, W. Li, U.S. Application No. 20010036481, (November 1, 2001).
3. *Lipid-Protein-Sugar Particles for Drug Delivery*, D. Kohane, M. Lipp, R. Langer, U.S. Application No. 20020150621 (October 17, 2002).
4. *Particles for Inhalation Having Sustained Release Properties*, S. Basu, J. Hrkach, M. Lipp, K. Elbert, D. Edwards, U.S. Application No. 20030118513, (June 26, 2003).
5. *Particulate Compositions for Pulmonary Delivery,* R. Batycky, D. Edwards, M. Lipp, U.S. Application No. 20030129139 (July 10, 2003).

This is Exhibit 2 referred to in the Declaration of Michael M. Lipp, sworn this 9th day of February, 2005.

A Notary Public

CINDY A. SRAGG
Notary Public
My Commission Expires
January 23, 2009

GOODMAN and GILMAN's

The Pharmacological Basis of Therapeutics

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CHAPTER

33 ANTIHYPERTENSIVE AGENTS AND THE DRUG THERAPY OF HYPERTENSION

John G. Gerber and Alan S. Nies

Hypertension, defined as an elevation of systolic and/or diastolic blood pressures to above 140/90 mm Hg, afflicts up to 60 million people in the United States; it is thus the most common cardiovascular disease. Hypertension has been classified as "malignant," when it results in arteriolitis, or "benign" ("essential"). Untreated, malignant hypertension causes the death of 90% of patients within a year. Adequate treatment of this condition can lead to long-term survival, and controlled clinical trials were not necessary to prove the benefit of treatment in this disease (Harrington *et al.*, 1959). Based on the elevation of the diastolic pressure, benign hypertension can be subdivided into mild (diastolic pressure, 90 to 104 mm Hg), moderate (diastolic pressure, 105 to 114 mm Hg), and severe (diastolic pressure, ≥ 115 mm Hg). The terms "benign" and "essential" as applied to hypertension were based on the mistaken impression that such elevation of blood pressure was not dangerous, since it did not cause symptoms in most patients, and might even be required for normal perfusion. It is now known that benign hypertension is a major risk factor for stroke, congestive heart failure, and coronary artery disease. Since 1972, the national age-adjusted mortality rate for stroke has fallen 50% and the mortality rate for coronary heart disease has fallen 35%; these changes have been associated with national programs for the detection and treatment of hypertension (Shea *et al.*, 1985; Joint National Committee, 1988). Clinical trials have shown that control of hypertension reverses the risk of stroke and congestive heart failure associated with high blood pressure; however, the risk of coronary disease is not reversed as readily (MacMahon *et al.*, 1986). Thus, these studies indicate that hypertension is neither benign nor essential in the usual sense of these terms.

Some of the earliest randomized, double-blind, controlled clinical trials of drug therapy for any disease were begun in the early 1960s for hypertension (*see* MacMahon *et al.*, 1986; Robertson, 1987). As a result of studies such as those by the Veterans Administration Cooperative Study Group on Antihypertensive Agents (1967, 1970), a substantial and unequivocal benefit of drug treatment to reduce serious cardiovascular morbidity was shown for patients with severe hypertension. The benefit was largely confined to events that were known to be direct results of elevated pressure, including cerebrovascular accidents (CVA), congestive heart failure, dissecting aneurysm, and nephropathy. The studies were all too small and of too short a duration to demonstrate a reduction of mortality, except for one study of patients who had recovered from a CVA, in which antihypertensive therapy enhanced survival related to a reduction of fatal cerebral reinfarction (Carter, 1970).

Subsequent studies of patients with mild-to-moderate hypertension have shown that antihypertensive therapy reduces the incidence of CVA; when the results from several trials were pooled, it was apparent that mortality from all causes was reduced, primarily because of a large decrease in the incidence of fatal stroke (MacMahon *et al.*, 1986). However, pooled data from all of the controlled studies suggest only a small trend to reduce the risk of coronary heart disease. The reasons for this are unknown but include the possibilities that 1) the study population was too small, 2) the follow-up period was too short, 3) active treatment in the "control" group of patients reduced the power of the studies, and 4) the drugs had adverse effects that contributed to the development of coronary disease and thus offset some of the benefits of reducing blood pressure (MacMahon *et al.*, 1986; Kaplan, 1988a). The drugs used for most of the trials were thiazide-like diuretics with the subsequent addition of a sympatholytic drug (in the United States usually reserpine). Various trials comparing β-adrenergic blocking agents with diuretics have produced mixed results, and the capacity of β-adrenergic antagonists to prevent coronary events in any group of hypertensive patients remains uncertain (The IPPPSH Collaborative Group, 1985; Medical Research Council Working Party, 1985;

1988; Wilhelmsen *et al.*, 1987; Wikstrand *et al.*, 1988).

In all of the studies of mild-to-moderate hypertension, the benefits of therapy were more obvious for patients with diastolic pressures ≥ 105 mm Hg than for those with diastolic pressures of 90 to 104 mm Hg; however, based on all of the data, it seems likely that antihypertensive drug therapy benefits all patients with diastolic pressures ≥ 95 mm Hg. Although patients with diastolic pressures of 90 to 94 mm Hg are certainly at higher risk of developing cardiovascular disease than are individuals with normal blood pressure, the benefit from drug therapy is less clear, and treatment must be individualized (*see* below). Increased mortality from coronary heart disease also appears to be caused by excessive lowering of arterial blood pressure, perhaps owing to the production of myocardial ischemia in patients who have a critical narrowing of the coronary arteries (Cruickshank, 1988). A challenge for future therapeutic trials is to determine whether the newer therapies are superior to previous approaches in reducing the risk of coronary heart disease in hypertensive patients, particularly in those with mild-to-moderate hypertension.

I. Pharmacology of Specific Antihypertensive Agents

Drugs (and physiological control mechanisms) influence arterial blood pressure at four effector sites—the resistance vessels (arterioles), the capacitance vessels (veins), the heart, and the kidneys—and they do so by several mechanisms (Table 33–1). Many of the antihypertensive drugs that affect adrenergic receptors, autonomic ganglia, the renin–angiotensin system, Ca^{2+} channels, and Na^+ and water balance have been discussed in detail in Chapters 9, 11, 28, 31, and 32. The pharmacology of antihypertensive agents that are not discussed elsewhere is presented here; in addition, the properties of all of the major drugs that are particularly relevant to their use in hypertension are reviewed, and an overview of the therapy of hypertension is provided.

The hemodynamic consequences of long-term treatment with antihypertensive agents are presented in Table 33–2, which also provides a framework for potential complementary effects of concurrent therapy with two or more drugs. The simultaneous use of drugs with similar mechanisms of action and hemodynamic effects often produces little additional benefit. However, concurrent use of drugs from different classes is a common strategy to achieve effective control of blood pressure with a tolerable burden of adverse effects.

Table 33–1. CLASSIFICATION OF ANTIHYPERTENSIVE DRUGS BY THEIR PRIMARY SITE OR MECHANISM OF ACTION

A. *Diuretics* (Chapter 28)
   1. Thiazides and related agents (hydrochlorothiazide, chlorthalidone, *etc.*)
   2. Loop diuretics (furosemide, bumetanide, ethacrynic acid)
   3. Potassium-sparing diuretics (triamterene, spironolactone, amiloride)

B. *Sympatholytic Drugs* (Chapters 9, 11, 33)
   1. Centrally acting agents (methyldopa, clonidine, guanabenz, guanfacine)
   2. Ganglionic blocking agents (trimethaphan)
   3. Adrenergic neuron blocking agents (guanethidine, guanadrel, reserpine)
   4. β-Adrenergic antagonists (propranolol, metoprolol, *etc.*)
   5. α-Adrenergic antagonists (prazosin, phenoxybenzamine, phentolamine)
   6. Mixed antagonists (labetalol)

C. *Vasodilators* (Chapter 33)
   1. Arterial (hydralazine, minoxidil, diazoxide)
   2. Arterial and venous (nitroprusside)

D. *Calcium Channel Blockers* (Chapter 32) (verapamil, diltiazem, nifedipine, nicardipine, nitrendipine)

E. *Angiotensin Converting Enzyme Inhibitors* (Chapter 31) (captopril, enalapril, lisinopril)

DIURETICS

One of the earliest strategies for the management of hypertension was to alter Na^+ balance by restriction of salt in the diet. Long-term alteration of Na^+ balance with drugs became practical in the 1950s with the development of the orally active benzothiadiazine (thiazide) diuretics (*see* Chapter 28). These agents and the related phthalimidine derivatives (*e.g.*, chlorthalidone) have become the mainstay of antihypertensive regimens. Not only do such diuretics have antihypertensive effects when used alone, they enhance the efficacy of virtually all other antihypertensive drugs.

The exact mechanism for reduction of arterial blood pressure by diuretics is not certain. The drugs first decrease extracellular volume and car-

Table 33–2. HEMODYNAMIC EFFECTS OF LONG-TERM ADMINISTRATION OF ANTIHYPERTENSIVE AGENTS *

	HEART RATE	CARDIAC OUTPUT	TOTAL PERIPHERAL RESISTANCE	PLASMA VOLUME	PLASMA RENIN ACTIVITY	RENAL BLOOD FLOW
Diuretics	↔	↔	↓	↧	↑	↧
Sympatholytic agents						
Centrally acting	↧	↧	↓	↥	↧	↧
Adrenergic neuron blockers	↧	↓	↓	↑	↥	↧
α Blockers	↥	↥	↓	↥	↔	↔
β Blockers						
No ISA †	↓	↓	↔	↥	↓	↧
ISA	↔	↔	↓	↥	↧	↧
Arteriolar vasodilators	↑	↑	↓	↑	↑	↥
Ca^{2+}-channel blockers	↓ or ↑	↥	↓	↔	↥	↥
Angiotensin converting enzyme inhibitors	↔	↔	↓	↔	↑	↥

* Changes are indicated as follows: ↑, increased; ↓, decreased; ↥, increased or no change; ↧, decreased or no change; ↔, unchanged.

† ISA, intrinsic sympathomimetic activity.

diac output. However, the hypotensive effect is maintained during long-term therapy because of reduced vascular resistance; cardiac output returns to pretreatment values and extracellular volume remains somewhat reduced. Because of the persistent reduction in vascular resistance, some investigators have postulated that the diuretics have a direct effect on vascular smooth muscle that is independent of their saluretic effect. However, substantial data indicate that this is not the case. Thus, anephric patients and nephrectomized animals do not show a reduction in blood pressure when given diuretics (Bennett *et al.*, 1977); a high salt intake or an infusion of saline (but not dextran) to counteract the net negative Na^+ balance produced by diuretics reverses the antihypertensive effect; during effective therapy plasma volume remains about 5% below pretreatment values and the plasma renin activity remains elevated, indicating a persistent small reduction in body Na^+ (Shah *et al.*, 1978); diuretics do not relax vascular smooth muscle *in vitro;* and the hemodynamic effects of the diuretics to reduce vascular resistance are reproduced by restriction of salt (Freis, 1983).

Potential mechanisms for reduction of vascular resistance by a persistent, albeit small, reduction in body Na^+ include a decrease in interstitial fluid volume; a fall in smooth muscle Na^+ concentration that may secondarily reduce intracellular Ca^{2+} concentration, such that the cells are more resistant to contractile stimuli; and a change in the affinity and response of cell surface receptors to vasoconstrictor hormones (Insel and Motulsky, 1984).

Benzothiadiazines and Related Compounds

Thiazides and related compounds comprise the most frequently used antihypertensive agents in the United States. These drugs have a similar pattern of pharmacological effects and are generally interchangeable with appropriate adjustment of dosage (*see* Chapter 28). The hypotensive effect of thiazides occurs at low doses (*e.g.*, 25 mg of hydrochlorothiazide or equivalent) that produce a small natriuretic effect; increasing the dose above the equivalent of 50 mg of hydrochlorothiazide per day usually will not increase the antihypertensive effect unless the patient is on a high-salt diet, in which case the lower dose may not produce a net loss of Na^+ (Materson *et al.*, 1978; McVeigh *et al.*, 1988). Larger doses of the thiazides cause obvious diuresis, increased loss of K^+ in the urine, more metabolic abnormalities (hypokalemia,

PLASMA RENIN ACTIVITY	RENAL BLOOD FLOW
↑	↧
↧	↧
↥	↧
↔	↔
↓	↧
↧	↧
↑	↥
↥	↥
↑	↥

decr or no change; ↔,

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(Materson *et al.*,
88). Larger doses
obv , diuresis,
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(hypokalemia,

hyperuricemia, hyperlipoproteinemia, and hyperglycemia), and symptoms that can cause poor patient compliance. The need for large doses of the thiazides can be avoided by modest restriction of Na^+ to a daily intake of 70 to 100 mmol; strict salt restriction is not necessary or desirable. Since the degree of K^+ loss relates to the amount of Na^+ delivered to the distal tubule, modest restriction of Na^+ can also minimize the production of hypokalemic alkalosis. Thiazide-like drugs are not effective as diuretics or antihypertensive agents in patients who have a glomerular filtration rate below 30 ml/min. One exception is metolazone, which retains efficacy in patients with this degree of renal failure.

Most patients will respond to thiazides within 2 to 4 weeks, although a minority will not achieve maximal reduction of arterial pressure for up to 12 weeks on a given dose. Therefore, doses should not be increased more often than every 2 to 4 weeks. The average response to a thiazide is a reduction of blood pressure of 20/10 mm Hg, but this is variable among patients. Although the blood pressure of patients who have suppressed plasma renin activity is almost uniformly sensitive to a thiazide, many other patients also respond. There is no way to predict the antihypertensive response from the duration or severity of the hypertension in a given patient, although thiazides are less likely to be effective as sole therapy in patients with severe hypertension. Since the effect of a thiazide is additive with that of other antihypertensive drugs, combination regimens that include a thiazide are common and rational. Thiazides also have the advantage of minimizing the retention of salt and water that is commonly caused by vasodilators and some sympatholytic drugs. If thiazides are not effective at a low dose, it is more rational either to substitute a different drug or to add a second drug than to increase the dose of thiazide above the equivalent of 50 mg of hydrochlorothiazide per day (which enhances the probability of unwanted effects).

Toxicity and Precautions. The adverse effects of diuretics are discussed in Chapter 28. However, because antihypertensive therapy is continued for many years in patients who often have no symptoms of disease, the adverse effects are particularly important in determining patient compliance. In addition, metabolic effects that are of little consequence during short-term therapy cause concern in the long term. There is usually no obvious consequence of thiazide-induced hyperuricemia, although gout occurs on occasion. Similarly, hyperglycemia is often minimal, but an occasional patient with adult-onset diabetes may decompensate when exposed to a thiazide. More problematic are the consequences of hypokalemia and hyperlipoproteinemia. Concerns have been raised by studies showing that effective antihypertensive therapy with thiazides has not produced the expected benefit of a reduced incidence of coronary heart disease (Multiple Risk Factor Intervention Trial Research Group, 1982; Kaplan 1988a). Hypokalemia may cause arrhythmias, and the thiazide-induced increase of cholesterol in low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) may enhance coronary atherosclerosis. Although these worries are appropriate, there is no substantial evidence to justify them (Freis, 1986). No study has shown hypokalemia to be closely linked to ventricular irritability in patients who have no evidence of overt heart disease other than left ventricular hypertrophy. However, this is not to suggest that hypokalemia is necessarily benign in hypertensive patients. Hypokalemia may account for some of the disturbances in glucose metabolism associated with thiazides, as well as symptoms of weakness and fatigue. Additionally, high-risk patients who have symptomatic coronary disease, congestive heart failure, and particularly those who are taking digitalis should be protected from hypokalemia. However, in the majority of otherwise healthy hypertensive patients, the mild hypokalemia that results from diuretics is of little clinical consequence.

Hypokalemia can be minimized in all patients by the use of low doses of the diuretic and modest dietary restriction of Na^+. In high-risk patients, supplementation with KCl or the use of a K^+-sparing diuretic in combination with a thiazide may be required. K^+-sparing diuretics are somewhat more effective than K^+ supplements in restoring plasma concentrations of K^+ to normal when hypokalemia already exists (Morgan and Davidson, 1980). The use of dietary means to replace K^+ has the disadvantages of high cost, the potential for excessive caloric intake, and the lack of sufficient Cl^- to correct the metabolic alkalosis. For in-

stance, bananas, a frequently prescribed source of K^+, have only about 1 mmol of K^+ per 2.5 cm of banana, and the usual replacement dose of K^+ is from 20 to 40 mmol per day.

The increase in LDL- and VLDL-cholesterol caused by diuretics is about 5 to 10%, with considerable intersubject variability. Long-term studies have suggested that the increase in lipids wanes with time and may return to baseline after 1 to 2 years of therapy, but this is not entirely clear (Fries, 1986; Lardinois and Neuman, 1988). Because of apprehension about the potential cardiovascular toxicity of the thiazides and the availability of many newer effective antihypertensive drugs, recommendations for initial drug therapy for hypertensive patients now include many drugs other than diuretics. However, there are insufficient data to determine whether these other drugs provide any additional benefit to reduce the incidence of coronary heart disease in hypertensive patients.

All of the thiazide-like drugs cross the placenta, but they have not been found to have direct adverse effects on the fetus. However, if administration of a thiazide is begun during pregnancy, there is a risk of transient volume depletion that may result in placental hypoperfusion. Since the thiazides appear in breast milk, they should be avoided by nursing mothers.

Other Diuretic Antihypertensive Agents

The thiazide-type diuretics are more effective antihypertensive agents than are the loop diuretics, such as furosemide and bumetanide, in patients who have normal renal function (Ram *et al.*, 1981). This differential effect is most likely related to the short duration of action of loop diuretics, such that a single daily dose does not cause a significant net loss of Na^+ for an entire 24-hour period. The spectacular efficacy of the loop diuretics in producing a rapid and profound natriuresis is a potential detriment for the treatment of hypertension. When a loop diuretic is given twice daily, the amount of natriuresis can be excessive and lead to more side effects than does a slower-acting, milder thiazide diuretic. The loop diuretics produce hypercalciuria, rather than the hypocalciuria associated with the thiazides. However, the other metabolic consequences of the thiazides are shared with the loop diuretics, including hypokalemia, hyperuricemia, glucose intolerance, and potentially adverse effects on plasma concentrations of lipids. Loop diuretics may be particularly useful in patients with azotemia. Some hypertensive patients with refractory edema may require the concurrent use of a thiazide and a loop diuretic, but such combinations have the potential to produce severe derangements in electrolyte balance and must be used with extreme caution (Wollam *et al.*, 1982).

Although spironolactone in doses up to 100 mg per day is equivalent to hydrochlorothiazide in its hypotensive effect (Jeunemaitre *et al.*, 1988), higher doses produce an unacceptable incidence of side effects (Schrijver and Weinberger, 1979). Spironolactone may be particularly useful for individuals with clinically significant hyperuricemia, hypokalemia, or glucose intolerance, and it is the agent of choice for management of primary aldosteronism. In contrast to thiazide diuretics, spironolactone does not affect plasma concentrations of Ca^{2+} or glucose. The effects of spironolactone on plasma lipids have not been studied extensively, but data indicate that the changes in triglycerides, LDL-cholesterol, and total cholesterol are less than those seen with the thiazides. However, spironolactone may decrease the concentration of HDL-cholesterol (Falch and Schreiner, 1983). The other potassium-sparing diuretics, triamterene and amiloride, are used primarily to reduce the kaliuresis and potentiate the hypotensive effect of a thiazide (De Carvalho *et al.*, 1980; Multicenter Diuretic Cooperative Study Group, 1981). These agents should be used cautiously with frequent measurements of K^+ concentrations in plasma in patients predisposed to hyperkalemia and in patients receiving K^+ supplements or K^+-containing "salt substitutes." Renal insufficiency is a relative contraindication to the use of K^+-sparing diuretics.

Diuretic-Associated Drug Interactions. Since the antihypertensive effects of diuretics are frequently additive with those of other antihypertensive agents, a diuretic is commonly used in combination with other drugs. The K^+- and Mg^{2+}-depleting effects of the thiazide-like and loop diuretics can potentiate arrhythmias that arise from digitalis toxicity. Corticosteroids can amplify the hypokalemia produced by the diuretics. All diuretics can decrease the clearance of Li^+, resulting in increased plasma concentrations of Li^+ and potential toxicity (Amdisen, 1982). Nonsteroidal antiinflammatory drugs that inhibit the synthesis of prostaglandins reduce the antihypertensive effects of diuretics. It is not known if this interaction is due to Na^+ retention as a result of blockade of the natriuretic effect of the diuretic by the antiinflammatory agent or whether the effect is related to inhibition of vascular synthesis of prostaglandins (Webster, 1985). Nonsteroidal antiinflammatory drugs, β-adrenergic receptor antagonists, and angiotensin converting enzyme inhibitors reduce plasma concentrations of aldosterone and can potentiate the hyperkalemic effects of a K^+-sparing diuretic.

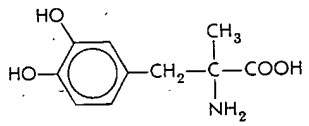
SYMPATHOLYTIC AGENTS

Since the demonstration in 1940 that bilateral excision of the thoracic sympathetic

chain could lower blood pressure, the search for effective, chemical sympatholytic agents has been intensive. Many compounds were tolerated poorly because they produced symptomatic orthostatic hypotension, sexual dysfunction, diarrhea, and fluid retention with subsequent reduction of the antihypertensive effect. However, newer agents and rational combinations of these drugs with diuretics and vasodilators have overcome many of these difficulties. The subgroups of sympatholytic agents are shown in Table 33–1.

METHYLDOPA

Introduced to clinical medicine in 1963, methyldopa was originally synthesized as an analog of 3,4-dihydroxyphenylalanine (DOPA) that can inhibit L-aromatic amino acid (DOPA) decarboxylase, an enzyme required for the biosynthesis of catecholamines (*see* Chapter 5). The structural formula of methyldopa, which is marketed as the L-isomer, is as follows:



Methyldopa

Locus and Mechanism of Action. When methyldopa was discovered to have antihypertensive actions, the mechanism was thought to be related to inhibition of catecholamine synthesis, with resultant inhibition of peripheral sympathetic function. This theory was subsequently shown to be incorrect; it was replaced by the "false neurotransmitter" theory (*see* Chapter 10) after the finding that methyldopa was metabolized in adrenergic neurons to methyldopamine and methylnorepinephrine. According to this theory, a false transmitter (a substance not normally present in neurons) accumulates in the same sites as the physiological transmitter and is released by the same stimuli that release the true transmitter. Although methylnorepinephrine fulfills these requirements for a false neurotransmitter, it is nearly as potent as norepinephrine and thus could not produce sufficient diminution of peripheral sympathetic function to explain the antihypertensive effects of methyldopa. Subsequently, methyldopa was found to be metabolized to false transmitters in adrenergic neurons in the brain, and this central effect is now thought to be responsible for the antihypertensive effects of the drug (Bobik *et al.*, 1986; Reid, 1986).

In animals, the hypotensive effect of methyldopa is blocked by DOPA decarboxylase inhibitors that have access to the brain but not by inhibitors that are excluded from the central nervous system (CNS). The hypotensive action is also abolished by inhibitors of dopamine β-hydroxylase and by centrally acting α-adrenergic receptor antagonists. These findings have led to the conclusion that methyldopa must be metabolized in the CNS to methylnorepinephrine, which produces its antihypertensive effect by stimulating α_2-adrenergic receptors in the brainstem; such stimulation results in decreased sympathetic outflow from the CNS.

However, not all data support the hypothesis that methylnorepinephrine is the major mediator of hypotension after the administration of methyldopa, since neither concentrations of methylnorepinephrine in the CNS nor turnover of methylnorepinephrine is closely correlated with the fall in blood pressure. Epinephrine-containing neurons have recently been described in regions of the CNS that are apparently important for regulation of blood pressure (Ward-Routledge and Marsden, 1988). Methyldopa can be metabolized to methylepinephrine both in the adrenal medulla and in the brain, and methylepinephrine is more potent than methylnorepinephrine in reducing arterial pressure when injected into the cerebral ventricles of animals. However, the amount of methylepinephrine actually formed from methyldopa in the CNS is probably too small to have pharmacological effects. Nonetheless, methyldopa depletes epinephrine from brainstem nuclei, raising the possibility that this action may have some relationship to the antihypertensive effects of methyldopa (Tung *et al.*, 1988).

Pharmacological Effects. Methyldopa reduces vascular resistance without causing much change in cardiac output or heart rate in younger patients with uncomplicated essential hypertension. In older patients, however, cardiac output may be decreased as a result of a reduction in heart rate and stroke volume; this is secondary to relaxation of veins and a reduction in preload. The fall in arterial pressure is maximal

6 to 8 hours after an oral or intravenous dose. Although the decrease in supine blood pressure is less than that in the upright position, symptomatic orthostatic hypotension is less common with methyldopa than with drugs that act exclusively on peripheral adrenergic neurons or autonomic ganglia. Renal blood flow is maintained, and renal function is unchanged during treatment with methyldopa.

Plasma concentrations of norepinephrine fall in association with the reduction in arterial pressure, and this reflects the decrease in sympathetic tone. Renin secretion is also reduced by methyldopa, but this is not a major effect of the drug and is not necessary for its hypotensive effects. Salt and water are often gradually retained with prolonged use of methyldopa, and this tends to blunt the antihypertensive effect. This has been termed "pseudotolerance," and it can be overcome with concurrent use of a diuretic. Of interest, treatment with methyldopa may reverse left ventricular hypertrophy within 12 weeks without any apparent relationship to the degree of change of arterial pressure (Fouad *et al.*, 1982).

Absorption, Metabolism, and Excretion. Since methyldopa is a prodrug that is metabolized in the brain to the active form, its concentration in plasma has less relevance for its effects than is true for many other drugs. When administered orally, methyldopa is absorbed by an active amino acid transporter. Peak concentrations in plasma occur after 2 to 3 hours. The drug is distributed in a relatively small apparent volume (0.4 liter/kg) and is eliminated with a half-life of about 2 hours. The transport of methyldopa into the CNS is apparently also an active process (Bobik *et al.*, 1986). Methyldopa is excreted in the urine primarily as the sulfate conjugate (50 to 70%) and as the parent drug (25%). The remaining fraction is excreted as other metabolites, including methyldopamine, methylnorepinephrine, and O-methylated products of these catecholamines (Campbell *et al.*, 1985). The half-life of methyldopa is prolonged to 4 to 6 hours in patients with renal failure.

In spite of its rapid absorption and short half-life, the peak effect of methyldopa is delayed for 6 to 8 hours, even after intravenous administration, and the duration of action of a single dose is usually about 24 hours; this permits once or twice daily dosing (Wright *et al.*, 1982). The discrepancy between the effects of methyldopa and the measured concentrations of the drug in plasma is most likely related to the time required for transport into the CNS, conversion to the active metabolites, and removal of these metabolites from the brain. Patients with renal failure are more sensitive to the antihypertensive effect of methyldopa, but it is not known if this is due to alteration in excretion of the drug or to an increase in transport into the CNS.

Preparations, Routes of Administration, and Dosage. *Methyldopa* (ALDOMET, others) is available in oral tablets containing 125, 250, or 500 mg and in an oral suspension (50 mg/ml). The usual initial dose is 250 mg twice daily, and there is little additional effect with doses above 2 g per day. Administration of a single daily dose of methyldopa at bedtime minimizes sedative effects, but administration twice daily may be required for some patients. A parenteral preparation of the ethyl ester of methyldopa, *methyldopate hydrochloride* (ALDOMET ESTER HYDROCHLORIDE) is also available (50 mg/ml). It is usually given by intermittent intravenous infusion of 250 to 1000 mg every 6 hours. The rate of deesterification of the methyldopate is variable among patients, and the doses given intravenously may deliver less methyldopa to the circulation than the same dose given orally.

Toxicity and Precautions. Methyldopa shares a number of side effects with other centrally acting α_2-adrenergic agonists (*see* Chapter 10 and below); the most frequent is sedation. This sedation may wane after several weeks of therapy but may recur with increases in dosage. Decreased mental acuity and forgetfulness are more subtle problems with an insidious onset that can incapacitate individuals who require a high degree of mental alertness. Symptomatic postural hypotension may occur, especially in patients who are depleted of salt and water as a result of aggressive use of diuretics. Other side effects that are related to the pharmacological effects in the CNS include dry mouth, nasal stuffiness, headaches, sleep disturbances, impotence, diarrhea, blurred vision, parkinsonian signs, bradycardia, carotid sinus hypersensitivity, first-degree heart block, and depression.

In addition to these annoying side ef-

fects, methyldopa is unique in producing a constellation of less common side effects that can be serious and require prompt discontinuation of the drug. These include hemolytic anemia, leukopenia, thrombocytopenia, hepatitis, red-cell aplasia, lupus-like syndromes, and hyperthermia that can mimic sepsis. At least 20% of patients who receive methyldopa for a year develop a positive Coombs' test that is due to autoantibodies directed against the Rh locus on the patient's erythrocytes. The development of Coombs' positivity *per se* is not an indication to stop therapy with methyldopa; however, 1 to 5% of these patients will develop a hemolytic anemia, which requires prompt discontinuation of the drug. The Coombs' test may remain positive for as long as a year after discontinuation of methyldopa, but the hemolytic anemia usually resolves in a matter of weeks. Severe hemolysis may be limited by treatment with corticosteroids.

The incidence of methyldopa-induced hepatitis is unknown, but about 5% of patients will have transient increases in transaminase activities in plasma. The development of hepatitis is usually heralded by symptoms of fatigue and anorexia that are similar to symptoms of viral hepatitis. Histologically, acute hepatitis due to methyldopa may be indistinguishable from viral hepatitis. Hepatic dysfunction is usually reversible with prompt discontinuation of the drug, but it will recur if methyldopa is taken again, and a few cases of fatal hepatic necrosis have been reported. Hepatitis may occur after long-term therapy with methyldopa, but it usually appears within 2 months of starting the drug. All patients receiving methyldopa should have serum transaminase activity measured monthly for the first 2 months and at the first sign or symptom of hepatitis, regardless of the duration of therapy. It is advisable to avoid the use of methyldopa in patients with hepatic disease.

A variety of rare toxic reactions include lichenoid and granulomatous skin eruptions, myocarditis, retroperitoneal fibrosis, pancreatitis, colitis, malabsorption, and hyperprolactinemia with or without gynecomastia and galactorrhea.

Adverse drug interactions that involve methyldopa are uncommon. Hypotension can be increased by the concurrent administration of diuretics, other antihypertensive agents, and general anesthetics. In experimental animals, tricyclic antidepressants interfere with the antihypertensive effect of methyldopa, but this has not been confirmed in controlled studies in man.

Therapeutic Uses. Methyldopa is an effective antihypertensive agent when given in conjunction with a diuretic. However, frequent side effects and the potential for immunological abnormalities and organ toxicity limit its usefulness.

CLONIDINE, GUANABENZ, AND GUANFACINE

The detailed pharmacology of the α_2-adrenergic agonists, clonidine, guanabenz, and guanfacine, is discussed in Chapter 10. These drugs stimulate α_2-adrenergic receptors in the brainstem, resulting in a reduction in sympathetic outflow from the CNS (Sattler and van Zwieten, 1967; Langer *et al.*, 1980). The decrease in plasma concentrations of norepinephrine is correlated directly with the hypotensive effect (Goldstein *et al.*, 1985; Sorkin and Heel, 1986). Patients who have had a spinal cord transection above the level of the sympathetic outflow tracts do not display a hypotensive response to clonidine (Reid *et al.*, 1977). At doses higher than those required to stimulate central α_2-adrenergic receptors, these drugs can activate α_2 receptors on vascular smooth muscle cells. This effect accounts for the initial vasoconstriction that is seen when overdoses of these drugs are taken, and it has been postulated to be responsible for the loss of therapeutic effect that is observed with high doses of these drugs (Frisk-Holmberg *et al.*, 1984; Frisk-Holmberg and Wibell, 1986).

Pharmacological Effects. The α_2-adrenergic agonists lower arterial pressure by an effect on both cardiac output and peripheral resistance. In the supine position, when the sympathetic tone to the vasculature is low, the major effect is to reduce both heart rate and stroke volume; however, in the upright position, when sympathetic outflow to the vasculature is normally increased, these drugs reduce vascular resistance. Some degree of ortho-

static hypotension always occurs because of a reduction in venous return (secondary to systemic venodilatation), but symptomatic postural hypotension is uncommon in the absence of volume depletion. Sympathetic reflexes are damped but not entirely inhibited, and the sympathetic responses that are associated with the use of arteriolar vasodilators such as hydralazine and minoxidil are blunted. However, the α_2-adrenergic agonists do not interfere with the hemodynamic response to exercise, and exercise-induced hypotension is unusual. These drugs do not reduce myocardial contractility directly, and the bradycardia that results from the decrease in cardiac sympathetic tone is rarely severe. Renal blood flow and glomerular filtration rate are maintained. Secretion of renin is often reduced, although it will respond to volume depletion or maintenance of an upright posture; there is no correlation between the hypotensive response and the effect on plasma renin activity. Retention of salt and water may occur with the α_2-adrenergic agonists, and it may be necessary to use a diuretic concurrently. Centrally acting α_2-adrenergic agonists have either no effect on plasma lipids or produce a slight reduction of total cholesterol, LDL-cholesterol, and triglycerides (Lardinois and Neuman, 1988).

When guanabenz was first introduced, there was considerable interest in observations that the drug could be natriuretic in experimental animals. However, studies in man have given variable results. With long-term therapy there is usually a small loss of weight with no clinically significant changes in salt and water balance, suggesting that the "pseudotolerance" (Na^+ retention) seen with methyldopa and guanethidine may not occur with guanabenz. Nonetheless, the antihypertensive effects of diuretics and guanabenz are additive. If individuals are given guanabenz after a salt load, the drug has a natriuretic effect, and a new steady-state of Na^+ balance is attained by 1 week. This short-term effect is thought to be related to a reduction in renal sympathetic stimulation, with a consequent reduction in Na^+ reabsorption in the proximal nephron (Gehr *et al.*, 1986). Guanabenz has also been shown to cause a water diuresis in some situations, which may be due to inhibition of the release and the renal actions of vasopressin (Strandhoy, 1985). Stimulation of renal α_2-adrenergic receptors by guanabenz may inhibit vasopressin-induced accumulation of cyclic AMP (Gellai and Edwards, 1988).

Toxicity and Precautions. Although the α_2-adrenergic agonists do not cause life-threatening adverse reactions, many patients experience annoying and sometimes intolerable side effects. Sedation and xerostomia occur in at least 50% of patients upon initiation of therapy with clonidine and guanabenz and in 25% of patients who receive guanfacine (Wilson *et al.*, 1986). Although these symptoms may diminish after several weeks of therapy, at least 10% of patients discontinue the drug because of persistence of these effects or because of impotence, nausea, or dizziness. The xerostomia may be accompanied by dry nasal mucosa, dry eyes, and parotid gland swelling and pain. Clonidine may produce a lower incidence of dry mouth and sedation when given transdermally, perhaps because high peak concentrations are avoided. Less common CNS side effects include sleep disturbances with vivid dreams or nightmares, restlessness, and depression. Cardiac effects related to the sympatholytic action of these drugs include symptomatic bradycardia in patients with dysfunction of the sinoatrial node and atrioventricular (AV) block in patients with AV nodal disease or in patients taking other drugs that depress the AV node. Fifteen to 20% of patients who receive transdermal clonidine may develop a contact dermatitis.

Sudden discontinuation of an α_2-adrenergic agonist may cause a withdrawal syndrome consisting of headache, apprehension, tremors, abdominal pain, sweating, and tachycardia. The arterial blood pressure may rise to levels above those that were present prior to treatment, but the syndrome may occur in the absence of an overshoot in pressure. Symptoms typically occur 18 to 36 hours after the drug is stopped, and they are associated with increased sympathetic discharge, as evidenced by elevated plasma and urine concentrations of catecholamines. The exact incidence of the withdrawal syndrome is not known, but it seems to be uncommon. It has been reported with all of the drugs of this class, but it may be milder with guanfacine, perhaps because of its longer half-life. Rebound hypertension has also been seen after discontinuation of transdermal administration of clonidine (Metz *et al.*,

1987). Patients who are maintained on a β-adrenergic antagonist after an α_2-adrenergic agonist is discontinued may have more severe hypertension during the withdrawal syndrome.

Treatment of the withdrawal syndrome depends on the urgency of reducing the arterial blood pressure. In the absence of hypertensive encephalopathy, patients can be treated with their usual dose of antihypertensive drug, which should reduce the pressure within 2 hours. If a more rapid effect is required, sodium nitroprusside or a combination of an α- and β-adrenergic blocker is appropriate. β-Adrenergic blocking agents should not be used alone in this setting, since they will accentuate the hypertension by allowing unopposed α-adrenergic vasoconstriction caused by the elevated circulating concentrations of epinephrine.

Because perioperative hypertension has been described in patients when clonidine was withdrawn the night before surgery, surgical patients who are being treated with an α_2-adrenergic agonist should either be switched to another drug prior to elective surgery or should receive their morning dose and/or transdermal clonidine prior to the procedure. All patients who receive one of these drugs should be apprised of the potential danger of discontinuing the drug abruptly, and patients known to be noncompliant with medications should not be given α_2-adrenergic agonists for hypertension.

Adverse drug interactions with α_2-adrenergic agonists are rare. Diuretics potentiate the hypotensive effect of these drugs in a predictable manner. A maximum effective dose of methyldopa will inhibit the hypotensive effect of clonidine, presumably because both drugs act in the same manner. Tricyclic antidepressants may inhibit the antihypertensive effect of clonidine, but the mechanism of this interaction is not known. It has been postulated that the α-adrenergic blocking effect of the antidepressants inhibits the action of clonidine in the CNS; this seems unlikely because the tricyclic antidepressants appear to have a low affinity for α_2-adrenergic receptors (U'Pritchard *et al.*, 1977).

Overdosage with an α_2-adrenergic agonist causes depression of the sensorium, transient hypertension followed by hypotension, bradycardia, and respiratory depression. The depressed respiration (with miosis) resembles the effects of an opioid. Treatment consists of ventilatory support, atropine or a sympathomimetic for bradycardia, and circulatory support with dopamine or dobutamine and intravenous fluids. Although systemic administration of an α-adrenergic antagonist that can enter the CNS may reverse the effects of the centrally-acting α_2 agonists, the use of supportive therapy seems more prudent.

Therapeutic Uses. The α_2-adrenergic agonists are usually used in conjunction with diuretics for the treatment of hypertension, but they may be effective when given alone; all of the drugs in this class are equally efficacious (Holmes *et al.*, 1983). These drugs are also effective in blunting the reflex increase in sympathetic activity produced by vasodilators, and they may be used instead of a β-adrenergic antagonist for this purpose.

Clonidine also has been used in hypertensive patients for the diagnosis of pheochromocytoma. The lack of suppression of the plasma concentration of norepinephrine to less than 500 pg/ml 3 hours after an oral dose of 0.3 mg of clonidine suggests the presence of such a tumor. A modification of this test, wherein overnight urinary excretion of norepinephrine and epinephrine is measured after administration of a 0.3-mg dose of clonidine at bedtime, may be useful when results based on plasma norepinephrine concentrations are equivocal (MacDougall *et al.*, 1988). Other uses for α_2-adrenergic agonists are discussed in Chapters 10, 14, and 22.

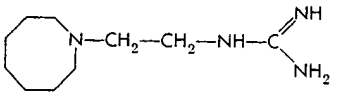
Ganglionic Blocking Agents

Ganglionic blocking agents are discussed in Chapter 9. These drugs are effective antihypertensive agents, but they are no longer used except for the short-term treatment of hypertension associated with dissecting aneurysm of the aorta and for the production of controlled hypotension during surgery. The most useful drug for aortic dissection is trimethaphan because it reduces both arterial blood pressure and the upslope of the arterial pressure wave in the aorta; the latter effect is important in slowing the propagation of the dissection. Trimethaphan camsylate is given by intravenous infusion at a rate of 0.3 to 5 mg/min. Hypotension is enhanced by having the patient in the Trendelenburg position; this increases venous pooling and results in reduced cardiac preload. The onset of the hypotensive effect is within 5 minutes, and the effect disappears within 15 minutes upon discontinuation of the infusion. Tachyphylaxis to the antihypertensive effect develops after 24 to 48 hours; this is partly due to expansion of plasma volume, and sensitivity can often be restored with diuresis. Trimethaphan produces a number of unwanted side effects related to ganglionic blockade, including paralytic ileus, bladder dysfunction, dry mouth, and blurred vision. Trimethaphan can produce res-

piratory arrest when given in doses greater than 5 mg/min, and such doses should be avoided.

GUANETHIDINE

Guanethidine is the prototype of drugs that specifically depress the activity of postganglionic sympathetic nerves. Guanethidine and related compounds contain a strongly basic moiety such as the guanidine group. The structure of guanethidine is as follows:



Guanethidine

Locus and Mechanism of Action. Guanethidine is uniquely targeted to the peripheral adrenergic neuron, where it inhibits sympathetic function. The drug reaches its site of action by active transport into the neuron, which is accomplished by the reuptake mechanism for norepinephrine (*see* Chapter 5). Once in the neuron guanethidine binds to storage vesicles. Initially, guanethidine produces sympathetic blockade by inhibiting the release of norepinephrine that normally follows nerve stimulation. Subsequently, guanethidine produces depletion of neuronal norepinephrine. In some ways guanethidine is reminiscent of a "false neurotransmitter," in that the drug is present in storage vesicles, it depletes the normal transmitter, and it can be released by stimuli that normally release norepinephrine. However, the fact that the sympathetic blockade is established well before significant depletion of norepinephrine takes place indicates that the false transmitter concept is not an adequate explanation for guanethidine's action (Shand *et al.*, 1973).

When given intravenously, guanethidine can initially release norepinephrine in an amount sufficient to increase arterial blood pressure. This does not occur with oral administration, since norepinephrine is released only slowly from the vesicles under this circumstance and is degraded within the neuron by monoamine oxidase. Nonetheless, because of the potential for norepinephrine release, guanethidine is contraindicated in patients with pheochromocytoma.

During adrenergic neuron blockade with guanethidine, effector cells become supersensitive to norepinephrine. The supersensitivity is similar to that produced by postganglionic sympathetic denervation.

Pharmacological Effects. Essentially all of the therapeutic and adverse effects of guanethidine result from sympathetic blockade (Woosley and Nies, 1976). A combination of venodilatation, which reduces cardiac preload, and inhibition of the cardiac sympathetic nerves results in a reduction in cardiac output. The arterioles do not respond to the reduction of cardiac output, since guanethidine blocks sympathetically mediated vasoconstriction. Thus, the arterial pressure is reduced modestly in the supine position when sympathetic activity is normally low, but the pressure can fall markedly during situations where sympathetic activation is increased, such as assumption of the upright posture, exercise, and depletion of plasma volume. Renal blood flow and glomerular filtration rate are modestly decreased during therapy with guanethidine, but this is without clinical consequence; renin secretion is not reduced. Plasma volume often becomes expanded, which may diminish the antihypertensive efficacy of guanethidine and require administration of a diuretic to restore the antihypertensive effect. Guanethidine does not enter the CNS and the drug does not affect brain function.

Absorption, Metabolism, and Excretion. The bioavailability of guanethidine is low and variable, and only 3 to 50% of an oral dose reaches the systemic circulation. The drug is rapidly transported to its intraneuronal site of action, from which it is eliminated with a half-life of 5 days. About 50% of the drug is metabolized, and the remainder is excreted unchanged in the urine. Because of its long half-life, guanethidine can be given once daily, and repeated daily doses will accumulate for at least 2 weeks.

Preparations, Routes of Administration, and Dosage. *Guanethidine monosulfate* (ISMELIN SULFATE) is available in 10- and 25-mg tablets for oral administration. The usual dose is 25 to 50 mg taken once daily, but the initial dose should be 10 mg per day. Guanethidine should always be given with a diuretic, and adjustments of dosage should be made no more often than every 2 weeks. Doses of up to 400 mg per day may be required. A loading regimen has been described for more rapid control of blood pressure in hospitalized patients (Shand *et al.*, 1975).

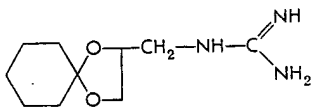
Toxicity and Precautions. Guanethidine produces undesirable effects that are related entirely to sympathetic blockade. Symptomatic hypotension during standing, exercise, ingestion of alcohol, and hot weather are the result of the lack of sympathetic compensation for these stresses. A general feeling of weakness is partially, but not entirely, related to postural hypotension. Rarely, guanethidine can precipitate congestive heart failure in patients with limited cardiac reserve as a result of the decrease in cardiac adrenergic tone and drug-induced fluid retention. Sexual dysfunction usually begins as delayed or retrograde ejaculation. Diarrhea may also occur and be sufficiently severe to limit the dose. Although diarrhea is commonly attributed to sympathetic blockade with parasympathetic predominance, the extent of the problem is not well correlated with the degree of sympathetic blockade, and other drugs that produce sympathetic blockade may cause less diarrhea.

Since guanethidine is actively transported to its site of action, drugs that block neuronal uptake of norepinephrine or displace norepinephrine from its storage sites will inhibit the effect of guanethidine. Such drugs include the tricyclic antidepressants, cocaine, chlorpromazine, ephedrine, phenylpropanolamine, and amphetamine (Michell *et al.*, 1970).

Therapeutic Uses. In the early 1970s guanethidine was a major antihypertensive drug for severely hypertensive patients; it was also used for patients who could not tolerate the CNS effects of methyldopa and reserpine. Many other drugs are now available that are as effective and that are much better tolerated than guanethidine; it is the rare patient who actually requires guanethidine.

GUANADREL

Guanadrel is another guanidine-containing adrenergic neuron blocking agent; its structure is as follows:



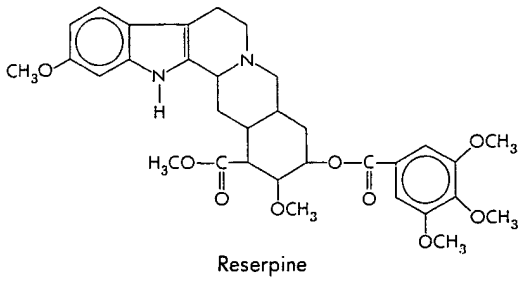
Guanadrel

Guanadrel and guanethidine act in the same way. The major difference between the two compounds is in their pharmacokinetic properties (Finnerty and Brogden, 1985). The bioavailability of guanadrel is high (85%), and the drug has an elimination half-life of 10 hours. Thus, guanadrel must be given twice daily to produce a sustained effect, and it accumulates to steady state rapidly. As a result of the short half-life and duration of action, claims have been made that the dose of guanadrel can be adjusted to avoid some of the side effects of guanethidine. In fact, most studies indicate that the efficacy and adverse effects of guanadrel and guanethidine are similar, with the exception of a lower incidence of diarrhea with guanadrel. Drug interactions with guanadrel are the same as with guanethidine.

Preparations, Routes of Administration, and Dosage. *Guanadrel sulfate* (HYLOREL) is available in 10- and 25-mg tablets for oral administration. The drug is given two or more times daily. The usual initial daily dose is 10 mg, and maintenance doses range from 20 to 75 mg per day.

RESERPINE

Reserpine is an alkaloid extracted from the root of *Rauwolfia serpentina* (Benth), a climbing shrub indigenous to India. Descriptions of the medicinal use of the root of this plant are present in ancient Hindu ayurvedic writings. "Modern" use of the whole root for the treatment of hypertension and psychoses was described in the Indian literature in 1931 (Sen and Bose, 1931). However, rauwolfia alkaloids were not used in Western medicine until the mid-1950s. Reserpine was the first drug that was found to interfere with the function of the sympathetic nervous system in man, and its use began the modern era of effective pharmacotherapy of hypertension. The structure of reserpine is as follows:



Reserpine

Locus and Mechanism of Action. Reserpine binds tightly to storage vesicles in central and peripheral adrenergic neurons, and the drug remains at such sites for prolonged periods of time (Giachetti and Shore, 1978). The storage vesicles are destroyed as a result of their interaction with reserpine, and nerve endings lose their ability to concentrate and store norepinephrine and dopamine. Catecholamines leak into the cytoplasm, where they are destroyed by intraneuronal monoamine oxidase, and little or no active transmitter is discharged from nerve endings when they are depolarized. A similar process occurs at storage sites for 5-hydroxytryptamine. Reserpine-induced depletion of biogenic amines correlates with evidence of sympathetic dysfunction and antihypertensive effects. Recovery of sympathetic function requires synthesis of new storage vesicles, which takes days to weeks after discontinuation of the drug. Since reserpine depletes amines in the CNS as well as in the peripheral adrenergic neuron, it is probable that its antihypertensive effects are related to both a central and a peripheral action; it is certain that many of the side effects of reserpine are related to its effects in the CNS.

Pharmacological Effects. Both cardiac output and peripheral vascular resistance are reduced during long-term therapy with reserpine. Orthostatic hypotension may occur but does not usually cause symptoms. Heart rate and renin secretion fall. Salt and water are retained, which commonly results in "pseudotolerance."

Absorption, Metabolism, and Excretion. Few data are available on the pharmacokinetic properties of reserpine because of the lack of an assay capable of detecting low concentrations of the drug or its metabolites. Reserpine that is bound to isolated storage vesicles cannot be removed by dialysis, indicating that the binding is not in equilibrium with the surrounding medium. Because of the irreversible nature of reserpine binding, the amount of drug in plasma is unlikely to bear any consistent relationship to drug concentration at the site of action. Reserpine is entirely metabolized, and none of the parent drug is excreted unchanged.

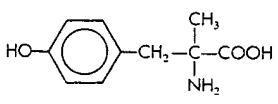
Preparations, Routes of Administration, and Dosage. Many preparations of rauwolfia and its derivatives are available. The powdered whole root of *Rauwolfia serpentina* (RAUDIXIN, others) is available in tablets containing 50 or 100 mg; 200 to 300 mg of this preparation is equivalent to 0.5 mg of reserpine. *Reserpine* (SERPASIL, others) is available in tablets containing 0.1, 0.25, and 1 mg. Reserpine is used once daily with a diuretic, and several weeks are necessary to achieve a maximum effect. The daily dose should be limited to 0.25 mg, and as little as 0.05 mg per day may be efficacious when a diuretic is also used.

Toxicity and Precautions. Most of the adverse effects of reserpine are due to its effect on the CNS. Sedation and inability to concentrate or perform complex tasks are the most common adverse effects. More serious is the occasional psychotic depression that can lead to suicide. Depression usually appears insidiously over many weeks or months and may not be attributed to the drug because of the delayed and gradual onset of symptoms. Reserpine must be discontinued at the first sign of depression, and the drug should never be given to patients with a history of depression. Depression appears to be uncommon, but not unknown, with doses of 0.25 mg per day or less. Other side effects include nasal stuffiness and exacerbation of peptic ulcer disease, which is uncommon with small oral doses. The literature contains epidemiological studies that link reserpine with breast cancer. It is nearly certain that these findings were the result of a bias in choosing patients, and recent data do not support the contention that reserpine is a risk factor for the development of breast cancer (Feinstein, 1988).

Therapeutic Uses. Reserpine was the sympatholytic drug used in the landmark Veterans Administration cooperative studies that demonstrated the beneficial effects of treatment of hypertension (Veterans Administration Cooperative Study Group on Antihypertensive Agents, 1967, 1970), but with the availability of newer drugs that are both effective and well tolerated, the use of reserpine has diminished because of its CNS side effects. However, in comparative studies, low doses of reserpine given concurrently with a diuretic were as well tolerated as combinations of a diuretic with propranolol or methyldopa. The major advantage of reserpine is that it is much less expensive than other antihypertensive drugs.

METYROSINE

Metyrosine is (−)-α-methyl-L-tyrosine. It has the following structure:



Metyrosine

Metyrosine is an inhibitor of tyrosine hydroxylase, the enzyme that catalyzes the conversion of tyrosine to DOPA; this is the rate-limiting step in catecholamine biosynthesis (*see* Chapter 5). At a dose of 1 to 4 g per day, metyrosine decreases catecholamine biosynthesis by 35 to 80% in patients with pheochromocytoma. The maximal decrease in synthesis occurs within several days, and the effect may be assessed by measurements of urinary catecholamines and their metabolites.

Metyrosine (DEMSER) has seen limited use as an adjuvant to phenoxybenzamine and other α-adrenergic blocking agents for the management of malignant pheochromocytoma (Brogden *et al.*, 1981). Metyrosine carries a risk of crystalluria, which can be minimized by maintaining a daily urine volume of more than 2 liters. Other adverse effects include sedation, extrapyramidal signs, diarrhea, anxiety, and psychic disturbances. Doses must be titrated carefully to achieve significant inhibition of catecholamine biosynthesis and yet minimize these substantive side effects.

β-ADRENERGIC ANTAGONISTS

β-Adrenergic receptor blocking drugs were not thought to have antihypertensive effects when they were first investigated. However, pronethalol, a drug that was never marketed, was found to reduce arterial blood pressure in hypertensive patients with angina pectoris. This antihypertensive effect was subsequently demonstrated for propranolol and all other β-adrenergic antagonists. The pharmacology of these drugs is discussed in Chapter 11; characteristics relevant to their use in hypertension will be described here.

Locus and Mechanism of Action. The precise mechanism for reduction of blood pressure by β-adrenergic antagonists is unknown, and it is likely that there are multiple modes of action. Among the theories that have been proposed are effects on renin secretion, cardiac output, adrenergic neuronal function, control of blood pressure in the CNS, baroreceptor sensitivity, and prostaglandin synthesis. Since all β-adrenergic antagonists are effective antihypertensive agents and since (+)-propranolol, which has little β-adrenergic receptor blocking activity, has no effect on blood pressure, the therapeutic effect is undoubtedly related to blockade of β-adrenergic receptors.

Pharmacological Effects. The β blockers vary in their lipid solubility, selectivity for

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NTAGONISTS

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ffects. The β blockers lu y, selectivity for the β_1-adrenergic receptor, presence of partial agonist or intrinsic sympathomimetic activity, and membrane-stabilizing properties. Regardless of these differences, all of the β-adrenergic antagonists are equally effective as antihypertensive agents. Drugs without intrinsic sympathomimetic activity produce an initial reduction in cardiac output and a rise in peripheral resistance with no net change in arterial pressure. In patients who respond with a reduction in blood pressure, peripheral resistance returns to pretreatment values in a few hours to a few days. It is this recovery of vascular resistance in the face of a persistently reduced cardiac output that accounts for the reduction in arterial pressure (van den Meiracker *et al.*, 1988). Drugs with intrinsic sympathomimetic activity produce less of an effect on resting heart rate and cardiac output, and the fall in arterial pressure is correlated with a fall in vascular resistance below pretreatment levels, probably because of stimulation of vascular β_2-adrenergic receptors that mediate vasodilatation.

Renal blood flow is reduced in the short term by most β-adrenergic antagonists, but reports of deterioration of renal function associated with long-term administration of these drugs are rare. Nevertheless, small reductions in renal plasma flow and glomerular filtration rate may persist, particularly with the nonselective drugs that block both β_1- and β_2-adrenergic receptors.

Toxicity and Precautions. The adverse effects of β-adrenergic blocking agents are discussed in Chapter 11. These drugs should be avoided in patients with reactive airway disease, congestive heart failure, or sinoatrial or atrioventricular nodal abnormalities. Patients with insulin-dependent diabetes are also better treated with other drugs. Although there have been concerns about the safety of the β-adrenergic antagonists for the treatment of hypertension during pregnancy, these drugs are effective and well tolerated, and controlled trials have not supported the initial fears about induction of premature labor, neonatal hypoglycemia, or small newborns.

β-Adrenergic antagonists without intrinsic sympathomimetic activity increase concentrations of triglycerides in plasma and lower those of HDL-cholesterol without changing total cholesterol concentrations. β-Adrenergic blocking agents with intrinsic sympathomimetic activity have little or no effect on blood lipids or increase HDL-cholesterol. The long-term consequences of these effects are unknown.

Sudden withdrawal of some β-adrenergic blockers can produce a withdrawal syndrome that is reminiscent of sympathetic hyperactivity; this can exacerbate the symptoms of coronary artery disease. Rebound hypertension to levels higher than those that existed before treatment has been noted with discontinuation of β-adrenergic antagonists in hypertensive patients (Houston and Hodge, 1988). Thus, β blockers should not be discontinued abruptly, except under close observation; dosage should be tapered over 10 to 14 days prior to discontinuation.

Nonsteroidal antiinflammatory drugs such as indomethacin can blunt the antihypertensive effect of propranolol and probably other β-adrenergic antagonists. This effect may be related to inhibition of vascular synthesis of prostacyclin, as well as to retention of Na^+ (Beckmann *et al.*, 1988).

Epinephrine can produce severe hypertension and bradycardia when a nonselective β-adrenergic receptor antagonist is present. This is due to the unopposed stimulation of α-adrenergic receptors when vascular β_2 receptors are blocked, and the bradycardia is the result of reflex vagal stimulation. Such "paradoxical" hypertensive responses to β-adrenergic antagonists have been observed in patients with hypoglycemia or pheochromocytoma or during withdrawal from clonidine or administration of epinephrine as a therapeutic agent.

Therapeutic Uses. The β-adrenergic receptor antagonists provide effective therapy for many cardiovascular and other diseases, and they are useful for all grades of hypertension. Despite marked differences in their pharmacokinetic properties, the antihypertensive effect of all the β blockers is of sufficient duration to permit once daily administration. Populations that have a lesser antihypertensive response to β blocking agents include the elderly and

blacks, but some individuals in these groups may have an excellent response. Patients who smoke were recently shown to have a lesser antihypertensive response to propranolol than do nonsmokers, but this may not be the case with a selective β_1 receptor antagonist such as metoprolol (The IPPPSH Collaborative Group, 1985; Medical Research Council Working Party, 1985; Wikstrand *et al.*, 1988). The β-adrenergic receptor antagonists do not usually cause retention of salt and water, and administration of a diuretic is not necessary to avoid edema or the development of tolerance. However, diuretics do have additive antihypertensive effects when combined with β blockers. The combination of a β-adrenergic antagonist, a diuretic, and a vasodilator is particularly effective. When minoxidil is the vasodilator, this combination can control the arterial pressure of most patients, even if they are resistant to other regimens.

α-Adrenergic Antagonists

The development of drugs that selectively block α_1-adrenergic receptors without affecting α_2-adrenergic receptors has added another group of effective antihypertensive agents. The pharmacology of these drugs is discussed in detail in Chapter 11. Prazosin and terazosin are the two agents that are available for the treatment of hypertension, and several additional congeners (*e.g.*, trimazosin and doxazosin) are being developed. Additionally, investigational drugs such as ketanserin, indoramin, and urapidil may owe a major portion of their antihypertensive effects to blockade of α_1-adrenergic receptors (Cubeddu, 1988).

Pharmacological Effects. Initially, prazosin and terazosin reduce arteriolar resistance and increase venous capacitance; this causes a sympathetically mediated reflex increase in heart rate and plasma renin activity. During long-term therapy, vasodilatation persists but cardiac output, heart rate, and plasma renin activity return to normal. Renal blood flow is unchanged during therapy with an α_1-adrenergic antagonist. Prazosin and terazosin can cause a variable amount of postural hypotension, depending on the plasma volume. Retention of salt and water occurs in many patients during continued administration of prazosin or terazosin, and this attenuates the postural hypotension. Administration of an α_1-adrenergic antagonist may reduce plasma concentrations of triglycerides and total and LDL-cholesterol and increase HDL-cholesterol. These potentially favorable effects on lipids persist when a thiazide-type diuretic is given concurrently. The long-term consequences of these drug-induced changes in lipids are unknown.

Toxicity and Precautions. The major precaution to be remembered when prazosin or terazosin is used for hypertension is the so-called first-dose phenomenon—symptomatic orthostatic hypotension that occurs within 90 minutes of the initial dose of the drug or when the dosage is increased rapidly. This effect may be seen in up to 50% of patients, and it is particularly likely to occur in patients who are already receiving a diuretic or a β-adrenergic antagonist.

Therapeutic Uses. Prazosin and terazosin can be used to treat hypertension of any degree, but these agents are usually not effective by themselves except in patients with mild-to-moderate hypertension. Diuretics and β-adrenergic antagonists enhance the efficacy of the α_1 blockers. Prazosin has been used in patients with pheochromocytoma, but it is not the drug of choice, since it is a short-acting competitive antagonist; in addition, vasoconstriction can still result from activation of unblocked vascular α_2-adrenergic receptors.

Combined α- and β-Adrenergic Antagonists

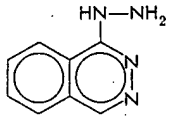
Labetalol (*see* Chapter 11) is an equimolar mixture of four stereoisomers. One isomer is an α_1-adrenergic antagonist (like prazosin), another is a nonselective β-adrenergic antagonist with partial agonist activity (like pindolol), and the other two isomers are inactive. The isomer that is the β-adrenergic antagonist is being developed as a separate drug (dilevalol) (Lund-Johansen, 1988). Labetalol lowers arterial pressure by reducing vascular resistance as a consequence of blockade of α_1-adrenergic receptors and stimulation of β_2 receptors. Cardiac output at rest is not reduced. Because of its capacity to block α_1 receptors, labetalol given intravenously can reduce pressure sufficiently rap-

idly to be useful for the treatment of hypertensive emergencies. Given over the long term, labetalol has efficacy and side effects similar to a combination of β- and α_1-adrenergic receptor antagonists; it also has the disadvantages that are inherent in fixed-dose combination products.

VASODILATORS

HYDRALAZINE

Hydralazine was one of the first orally active antihypertensive drugs to be marketed in the United States; however, the drug quickly lost its popularity because of unacceptable side effects and tachyphylaxis. With a better understanding of the compensatory cardiovascular responses that accompany use of arteriolar vasodilators, hydralazine was combined with sympatholytic agents and diuretics with greater therapeutic success. Numerous phthalazines have been synthesized in the hope of producing vasoactive agents, but only those with hydrazine moieties in the 1 or 4 position of the ring have vasodilatory activity (Reece, 1981). None of the analogs has any advantage over hydralazine. Hydralazine (1-hydrazinophthalazine) has the following structural formula:



Hydralazine

Locus and Mechanism of Action. Hydralazine causes direct relaxation of arteriolar smooth muscle. The mechanism of this effect is unclear, and numerous hypotheses have been put forth. Part of the vascular relaxation caused by hydralazine is dependent on the presence of the endothelium (Spokas *et al.*, 1983). In addition, nitric oxide can be generated from hydralazine *in vitro* (Kruszyna *et al.*, 1987). Since nitric oxide may be liberated from endothelium-derived relaxing factor (EDRF) or may itself be EDRF, the mechanism of action of hydralazine is similar to the mechanisms of action of EDRF, organic nitrates, and sodium nitroprusside (*see* Chapter 32). Hydralazine can also cause hyperpolarization of isolated arteries and interfere with mobilization of Ca^{2+} in vascular smooth muscle (Kreye, 1984). Hydralazine-induced vasodilatation is associated with stimulation of the sympathetic nervous system, which results in increased heart rate and contractility, increased plasma renin activity, and fluid retention; all of these effects counteract the antihypertensive effect of hydralazine. Although most of the sympathetic activity is due to a baroreceptor-mediated reflex, hydralazine may stimulate the release of norepinephrine from sympathetic nerve terminals and augment myocardial contractility directly (Azuma *et al.*, 1987).

Pharmacological Effects. Most of the effects of hydralazine are confined to the cardiovascular system. The decrease in blood pressure after administration of hydralazine is associated with a selective decrease in vascular resistance in the coronary, cerebral, and renal circulation, with a smaller effect in skin and muscle. Because of preferential dilatation of arterioles over veins, postural hypotension is not a common problem. Hydralazine lowers peripheral vascular resistance equally in the supine and upright positions. Although hydralazine lowers pulmonary vascular resistance, the increase in cardiac output can cause mild pulmonary hypertension. It is difficult to predict which patients will respond in this manner, but the increase in cardiac output can be attenuated by the use of β-adrenergic blocking agents.

Absorption, Metabolism, and Excretion. Hydralazine is well absorbed through the gastrointestinal tract, but the systemic bioavailability is low (16% in fast acetylators and 35% in slow acetylators). Since the acetylated compound is inactive, the dose necessary to produce a systemic effect is larger in fast acetylators. N-acetylation of hydralazine occurs in the bowel and/or the liver, and the rate of acetylation is genetically determined; about half of the people in the United States acetylate rapidly and half do so slowly. The half-life of hydralazine is 1 hour and the systemic clearance of the drug is about 50 ml/kg · min. Since the systemic clearance exceeds hepatic blood flow, extrahepatic metabolism must occur. Indeed, hydralazine rapidly combines with

circulating α keto acids to form hydrazones, and the major metabolite recovered from the plasma is hydralazine pyruvic acid hydrazone. This metabolite has a longer half-life than hydralazine, but it does not appear to be very active (Reece *et al.*, 1985). Although the rate of acetylation is an important determinant of the bioavailability of hydralazine, it does not play a role in the systemic elimination of the drug. This suggests that almost all acetylation of hydralazine occurs prior to the time that the drug reaches the systemic circulation. This pharmacokinetic profile is unusual, since other drugs that are metabolized by the genetically determined N-acetyltransferase (*e.g.*, isoniazid, dapsone, procainamide) have a rate of elimination that is dependent upon the acetylator phenotype.

The peak concentration of hydralazine in plasma and the peak hypotensive effect of the drug occur within 30 to 120 minutes of ingestion. Although its half-life in plasma is about an hour, the duration of the hypotensive effect can last as long as 12 hours. There is no clear explanation for this discrepancy.

Preparations, Routes of Administration, and Dosage. *Hydralazine hydrochloride* (APRESOLINE HYDROCHLORIDE, others) is available in 10-, 25-, 50-, and 100-mg tablets and in 1-ml ampules containing 20 mg of the drug. The usual oral dosage is 25 to 100 mg twice daily. Twice-daily administration of hydralazine is as effective as administration four times a day for control of blood pressure, regardless of acetylator phenotype. Hydralazine can be given intramuscularly or intravenously in doses of 20 to 40 mg when there is an urgent need to lower blood pressure.

Toxicity and Precautions. Two types of side effects occur after the use of hydralazine. The first, which are extensions of the pharmacological effects of the drug, includes headache, nausea, flushing, hypotension, palpitation, tachycardia, dizziness, and angina pectoris. In addition, if the drug is used alone, there may be salt retention with development of congestive heart failure. These symptoms were common during the early clinical use of hydralazine; because tachyphylaxis developed, the daily dose of the drug was frequently increased to 400 to 1000 mg. When combined with a β-adrenergic receptor blocker and a diuretic, hydralazine is better tolerated, although side effects such as headache are still commonly described and may necessitate discontinuation of the drug.

The second type of side effect is caused by immunological reactions, of which the drug-induced lupus syndrome is the most common. Administration of hydralazine can also result in an illness that resembles serum sickness, hemolytic anemia, vasculitis, and rapidly progressive glomerulonephritis. The mechanism of these autoimmune reactions is unknown, but hydralazine has recently been shown to inhibit methylation of DNA and induce self-reactivity in T cells (Cornacchia *et al.*, 1988).

The drug-induced lupus syndrome usually occurs after at least 6 months of continuous treatment with hydralazine, and its incidence is related to dose, sex, acetylator phenotype, and race (Perry, 1973). In one study, after three years of treatment with hydralazine drug-induced lupus occurred in 10.4% of patients who received 200 mg daily, 5.4% who received 100 mg daily, and none who received 50 mg daily (Cameron and Ramsay, 1984). The incidence is four times higher in women than in men, and the syndrome is seen more commonly in whites than in blacks. The rate of conversion to a positive antinuclear antibody test is faster in slow acetylators than in rapid acetylators, suggesting that the native drug or a nonacetylated metabolite is responsible. However, since the majority of patients with positive antinuclear antibody tests do not develop the drug-induced lupus syndrome, hydralazine need not be discontinued unless clinical features of the syndrome appear. These features are similar to those of other drug-induced lupus syndromes and consist mainly of arthralgia, arthritis, and fever. Pleuritis and pericarditis may be present, and pericardial effusion can occasionally cause cardiac tamponade. Discontinuation of the drug is all that is necessary for most patients with the hydralazine-induced lupus syndrome, but symptoms may persist in a few patients and administration of corticosteroids may be necessary.

Hydralazine can also produce a pyridoxine-responsive polyneuropathy. The mechanism appears to be related to the ability of hydralazine to combine with pyridoxine to form a hydrazone. This side effect is very unusual with doses up to 200 mg per day.

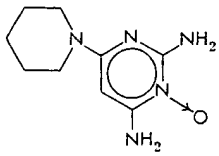
Therapeutic Uses. Hydralazine should be used for the treatment of hypertension only when the combination of a diuretic and a β-adrenergic antagonist does not control blood pressure adequately. Hydralazine should never be used as the sole drug for

the long-term treatment of hypertension because of the development of tachyphylaxis secondary to an increase in cardiac output and fluid retention. In addition, the drug should be used with the greatest of caution in elderly patients and in patients with coronary artery disease because of the possibility of precipitation of myocardial ischemia. The maximum recommended dose of hydralazine is 200 mg per day in order to avoid the drug-induced lupus syndrome. Slow acetylators show a better response to this dosage than do fast acetylators because of the greater bioavailability of the drug.

Hydralazine has been used widely to treat hypertension that occurs during pregnancy. However, the drug should be used cautiously during early pregnancy, since hydralazine can combine with DNA and cause a positive Ames test (Williams *et al.*, 1980). Parenteral administration of hydralazine has been used for the treatment of hypertensive emergencies. However, the hypotensive response to hydralazine given intramuscularly or intravenously is very unpredictable, and prolonged hypotension is not unusual even with intravenous doses as low as 10 mg. The drug is contraindicated for the short-term production of hypotension in patients with dissecting aortic aneurysm or in those with symptomatic ischemic heart disease.

MINOXIDIL

The discovery in 1965 of the hypotensive action of minoxidil was a significant advance in the treatment of hypertension, since the drug has proven to be efficacious in patients with the most severe and drug-resistant forms of hypertension. The chemical structure of minoxidil is as follows:



Minoxidil

Locus and Mechanism of Action. Minoxidil is not active *in vitro* but must be metabolized by hepatic sulfotransferase to the active molecule, minoxidil N-O sulfate (McCall *et al.*, 1983); the formation of this compound is a minor pathway in the metabolic disposition of minoxidil. Minoxidil sulfate relaxes vascular smooth muscle in isolated systems where the parent drug is inactive. The mechanism of this effect is incompletely understood, but there is mounting evidence that minoxidil sulfate increases the permeability of the cell membrane to K^+, with resultant hyperpolarization (Meisheri *et al.*, 1988).

Pharmacological Effects. Minoxidil produces arteriolar vasodilatation with essentially no effect on the capacitance vessels; the drug resembles hydralazine and diazoxide in this regard. Minoxidil increases blood flow to skin, skeletal muscle, the gastrointestinal tract, and the heart more than to the CNS. The disproportionate increase in blood flow to the heart may have a metabolic basis, in that administration of minoxidil is associated with a reflex increase in cardiac output and myocardial contractility. This compensatory increase in cardiac output can be as much as threefold to fourfold and is mediated by the sympathetic nervous system.

The effects of minoxidil on the kidney are complex. Minoxidil is a renal vasodilator, but systemic hypotension produced by the drug can occasionally decrease renal blood flow and worsen renal function. However, in the majority of patients who take minoxidil for the treatment of hypertension, renal function improves, especially if renal dysfunction is secondary to hypertension (Mitchell *et al.*, 1980). Minoxidil is a very potent stimulator of renin secretion; this effect is mediated by a combination of renal sympathetic stimulation and activation of the intrinsic renal mechanisms for regulation of renin release.

Absorption, Metabolism, and Excretion. Minoxidil is well absorbed from the gastrointestinal tract. Although peak concentrations of minoxidil in blood occur 1 hour after oral administration, the maximal hypotensive effect of the drug occurs later, possibly because formation of the active metabolite is delayed. Only about 20% of

the absorbed drug is excreted unchanged in the urine, and the main route of elimination is by hepatic metabolism. The major metabolite of minoxidil is the glucuronide conjugate at the N-oxide position in the pyrimidine ring. This metabolite is less active than minoxidil, but it persists longer in the body. The extent of biotransformation of minoxidil to its active metabolite, minoxidil N-O sulfate, has not been evaluated in man. Minoxidil has a half-life in plasma of 3 to 4 hours, but its duration of action is 24 hours or occasionally even longer. It has been proposed that persistence of minoxidil in vascular smooth muscle is responsible for this discrepancy. However, without knowledge of the pharmacokinetic properties of the active metabolite, an explanation for the prolonged duration of action cannot be given.

Preparation, Routes of Administration and Dosage. *Minoxidil* (LONITEN, others) is supplied in 2.5- and 10-mg tablets. The initial daily dose is usually 5 mg, which can be increased gradually to 40 mg in one or two daily doses. Although daily doses of up to 100 mg have been used, most patients require 40 mg or less (Dormois *et al.*, 1975).

Toxicity and Precautions. Minoxidil is well tolerated, even though use of the drug is confined to patients with severe hypertension. The side effects of minoxidil are predictable and can be divided into three major categories: fluid and salt retention, cardiovascular effects, and hypertrichosis.

Retention of salt and water results from increased proximal renal tubular reabsorption, which is in turn secondary to reduced renal perfusion pressure and to reflex stimulation of renal tubular α-adrenergic receptors. Similar antinatriuretic effects can be observed with the other arteriolar dilators (*e.g.*, diazoxide and hydralazine). Although administration of minoxidil causes increased secretion of renin and aldosterone, this is not an important mechanism for retention of salt and water in this case. Fluid retention can usually be controlled by the administration of a diuretic. However, thiazides may not be sufficiently efficacious, and it may be necessary to use a loop diuretic. This is especially true if the patient has any degree of renal dysfunction.

The majority of the cardiovascular effects of minoxidil are secondary to baroreceptor-mediated activation of the sympathetic nervous system. Increased cardiac contractility can be effectively counteracted with adequate doses of a β-adrenergic antagonist, but some increase in heart rate may persist because tachycardia is mediated in part by withdrawal of parasympathetic tone. Pulmonary hypertension may result from increased cardiac output (despite decreased pulmonary vascular resistance) or from salt and water retention with consequent congestive heart failure. Minoxidil should thus be used with caution in patients with ischemic heart disease. However, with adequate blockade of β-adrenergic receptors, the decrease in blood pressure and heart size associated with reduction of afterload can favorably affect myocardial oxygen demand in hypertensive patients. The flattened and inverted T waves observed in the electrocardiogram during initial therapy with minoxidil are not ischemic in origin. Pericardial effusion is a real but rare complication of minoxidil. Although more commonly described in patients with renal failure and congestive heart failure, pericardial effusion can occur in patients with normal cardiovascular and renal function. Asymptomatic pericardial effusion is not an indication for stopping minoxidil, but the situation should be monitored closely to avoid progression to tamponade. The effusion usually clears when the drug is discontinued, but it will recur if treatment with minoxidil is resumed (Reichgott, 1981).

Hypertrichosis, which occurs in all patients who receive minoxidil for an extended period, is particularly offensive to women. Growth of hair occurs on the face, back, arms, and legs. The cause of this side effect is unclear, but it may be secondary to enhanced cutaneous blood flow. Similar side effects have been described during long-term oral administration of diazoxide. Frequent shaving or depilatory agents can be used to manage this problem. Topical minoxidil (ROGAINE 2% solution) is now marketed for the treatment of male-pattern baldness. The topical use of minoxidil can cause measurable cardiovascular effects in some individuals (Leenen *et al.*, 1988).

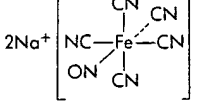
Other side effects of the drug are rare

and include rashes, Stevens–Johnson syndrome, glucose intolerance, serosanguinous bullae, formation of antinuclear antibodies, and thrombocytopenia.

Therapeutic Uses. Minoxidil is best reserved for the treatment of severe hypertension that responds poorly to other antihypertensive medications (Campese, 1981). It has been used successfully in the treatment of hypertension in both adults and children. Minoxidil should never be used alone; it must be given concurrently with a diuretic to avoid fluid retention and a sympatholytic drug (usually a β-adrenergic antagonist) to control reflex cardiovascular effects. The drug is usually administered either once or twice a day, but some patients may require more frequent dosage for adequate control of blood pressure.

Sodium Nitroprusside

Although sodium nitroprusside has been known since 1850 and its hypotensive effect in man was described in 1929, its safety and usefulness for the short-term control of severe hypertension were not demonstrated until the mid 1950s. Several investigators subsequently demonstrated that sodium nitroprusside was also effective in improving cardiac function in patients with left ventricular failure. The structural formula of sodium nitroprusside is as follows:



Sodium Nitroprusside

Locus and Mechanism of Action. The nitroso moiety of sodium nitroprusside is necessary for its vasodilatory action. When nitroprusside comes in contact with red blood cells, the molecule decomposes, releasing nitric oxide (Smith and Kruszyna, 1974). Nitric oxide is an unstable compound that causes vasodilatation and inhibits platelet aggregation by activating guanylate cyclase in vascular smooth muscle cells and platelets (*see* Chapter 32; Ignarro *et al.*, 1980). Recent evidence also suggests that nitric oxide is generated endogenously; as mentioned above, it may be the same as EDRF or be derived from EDRF (Moncada *et al.*, 1988).

Pharmacological Effects. Nitroprusside dilates both arterioles and venules, and the hemodynamic response to its administration results from a combination of venous pooling and reduced arterial impedance. Because of its effect on venules, sodium nitroprusside is a more effective hypotensive agent when the patient is upright. In subjects with normal left ventricular function, venous pooling affects cardiac output more than does the reduction of afterload; cardiac output thus tends to fall. In contrast, in patients with severely impaired left ventricular function and diastolic ventricular distention, the combination of venous pooling and the reduction of arterial impedance cause a rise in cardiac output.

Sodium nitroprusside is a nonselective vasodilator, and regional distribution of blood flow is little affected by the drug. In general, renal blood flow and glomerular filtration are maintained, and plasma renin activity increases. Unlike minoxidil, hydralazine, diazoxide, and other arteriolar vasodilators, administration of sodium nitroprusside usually causes only a modest increase in heart rate and an overall reduction in myocardial demand for oxygen.

Absorption, Metabolism, and Excretion. Sodium nitroprusside is an unstable molecule that decomposes under strongly alkaline conditions and when exposed to light. The drug must be given by continuous intravenous infusion to be effective. Its onset of action is within 30 seconds; the peak hypotensive effect occurs within 2 minutes, and when the infusion of the drug is stopped, the effect disappears within 3 minutes.

The breakdown of nitroprusside is probably initiated by its reduction. The ferrous ion of the drug reacts promptly with membrane-bound sulfhydryl groups of the vascular wall and erythrocytes to form an unstable nitroprusside radical, which then immediately dissociates into its components, cyanide and nitric oxide (Ivankovich *et al.*, 1978). Cyanide is further metabolized by liver rhodanase to thiocyanate, which is

eliminated almost entirely in the urine. The mean elimination half-time for thiocyanate is 3 days in patients with normal renal function, and it can be much longer in patients with renal insufficiency.

Preparation, Route of Administration, and Dosage. *Sodium nitroprusside* (NIPRIDE, NITROPRESS) is available in 2- or 5-ml vials that contain 50 mg. The contents of the vial should be dissolved in 2 to 3 ml of 5% dextrose in water. Addition of this solution to 250 to 1000 ml of 5% dextrose in water produces a concentration of 50 to 200 μg/ml. Because the compound decomposes in light, only fresh solutions should be used and the bottle should be covered with an opaque wrapping. The drug must be administered as a controlled, continuous infusion and the patient must be closely observed. The majority of hypertensive patients respond to an infusion of 0.5 to 1.5 μg/kg per minute. Higher rates of infusion are necessary to produce controlled hypotension in normotensive patients under surgical anesthesia. High rates of infusion of nitroprusside over a prolonged period can cause cyanide and/or thiocyanate poisoning. Patients who are receiving other antihypertensive medications usually require less nitroprusside to lower blood pressure. If infusion rates of 10 μg/kg per minute do not produce adequate reduction of blood pressure within 10 minutes, administration of nitroprusside should be stopped to minimize potential toxicity.

Toxicity and Precautions. The short-term side effects of nitroprusside are due to excessive vasodilatation with hypotension and the consequences thereof. Close monitoring of blood pressure and the use of a continuously variable-rate infusion pump will prevent an excessive hemodynamic response to the drug in the majority of the cases. Less commonly, toxicity may result from conversion of nitroprusside to cyanide and thiocyanate. Accumulation of cyanide can occur if sodium nitroprusside is infused at a rate greater than 2 μg/kg per minute. The limiting factor in the metabolism of cyanide appears to be the availability of sulfur-containing substrates in the body (mainly thiosulfate). The concomitant administration of sodium thiosulfate can prevent accumulation of cyanide in patients who are receiving higher than usual doses of sodium nitroprusside; the efficacy of the drug is unchanged (Schulz, 1984). The risk of thiocyanate toxicity increases when sodium nitroprusside is infused for more than 24 to 48 hours, especially if renal function is impaired. Signs and symptoms of thiocyanate toxicity include anorexia, nausea, fatigue, disorientation, and toxic psychosis. The plasma concentration of thiocyanate should be monitored during prolonged infusions of nitroprusside and should not be allowed to exceed 0.1 mg/ml. Rarely, excessive concentrations of thiocyanate may cause hypothyroidism by inhibiting iodine uptake by the thyroid gland. In patients with renal failure, thiocyanate can be removed readily by hemodialysis.

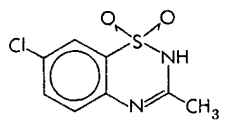
Nitroprusside can worsen arterial hypoxemia in patients with chronic obstructive pulmonary disease because the drug interferes with hypoxic pulmonary vasoconstriction and therefore promotes mismatching of ventilation with perfusion. Rebound hypertension may occur after abrupt cessation of short-term nitroprusside infusions (Packer *et al.*, 1979); this may be caused by persistently elevated concentrations of renin in the plasma.

Therapeutic Uses. Sodium nitroprusside is used primarily to treat hypertensive emergencies, but the drug can be used in many situations when short-term reduction of cardiac preload and/or afterload is desired. Thus, nitroprusside has been used to lower blood pressure during acute aortic dissection, to increase cardiac output in congestive heart failure, and to decrease myocardial oxygen demand after acute myocardial infarction. In addition, nitroprusside is the drug most often used to induce controlled hypotension during anesthesia in order to reduce bleeding in surgical procedures. In the treatment of acute aortic dissection, it is important to administer a β-adrenergic antagonist with nitroprusside, since reduction of blood pressure with nitroprusside alone can increase the rate of development of force by the heart, thereby enhancing propagation of the dissection.

DIAZOXIDE

Diazoxide was initially developed as an oral antihypertensive drug, but early clinical trials revealed unacceptable toxicity. At least 50% of patients displayed hyperglycemia, and 20% developed hypertrichosis. The drug was then marketed for parenteral use for the treatment of hypertensive emergencies, but sodium nitroprusside soon replaced diazoxide as the drug of choice for this indi-

cation. Diazoxide maintains a place in the treatment of hypertensive emergencies in situations in which accurate infusion pumps are not available and close monitoring of blood pressure is not feasible. The drug is a benzothiadiazine derivative, like the thiazide diuretics, but it does not cause diuresis, apparently because it lacks a sulfonamido group. Its structural formula is as follows:



Diazoxide

Mechanism of Action and Pharmacological Effects. Diazoxide hyperpolarizes arterial smooth muscle cells by activating ATP-sensitive K^+ channels; this causes relaxation of the vascular smooth muscle (Standen *et al.*, 1989). The effect of the drug *in vivo* is exclusively arteriolar, with negligible effect on capacitance vessels. Reflex activation of the sympathetic nervous system and retention of salt and water occur. Cardiac output may double from stimulation of heart rate and myocardial contractility. The avid retention of salt and water was thought to be secondary to a direct effect of diazoxide on renal-tubular function. However, studies in animals have never substantiated these claims, and in fact, direct infusion of diazoxide into the renal artery causes renal vasodilatation and diuresis (Brouhard *et al.*, 1981). More likely, the salt and water retention is a result of stimulation of renal sympathetic nerves and changes in intrarenal hemodynamics, as with other arteriolar vasodilators. Diazoxide increases coronary blood flow, and cerebral and renal blood flows are maintained by autoregulation. Renin secretion is enhanced, and the combination of an increased cardiac output, salt and water retention, and elevated concentrations of angiotensin II counteract the antihypertensive effects of diazoxide.

Absorption, Metabolism, Excretion. Although well absorbed orally, diazoxide is administered only intravenously for the treatment of severe hypertension. Approximately 20 to 50% of the drug is eliminated as such by the kidney, and the rest is metabolized in the liver to the 3 hydroxymethyl and 3 carboxy derivatives (Pruitt *et al.*, 1974). Although the plasma half-life of diazoxide is 20 to 60 hours, the duration of the hypotensive response to the drug is variable and can be as short as 4 hours or as long as 20 hours.

Preparation, Route of Administration, Dosage, and Therapeutic Use. *Diazoxide* (HYPERSTAT I.V.) is available for intravenous use in solutions containing 15 mg/ml. The main indication is for the treatment of hypertensive emergencies. Injection of an intravenous bolus lowers blood pressure within 30 seconds, and a maximum effect is achieved within 3 to 5 minutes. Because of the ease of administration and the rapid response, the drug can be used in emergency situations in which close monitoring of blood pressure is not feasible. Although initial recommendations were to administer a 300-mg bolus of diazoxide, excessive hypotension with resultant cerebral and cardiovascular damage has resulted from this practice. Hypotension can be minimized by the administration of a "minibolus" of 50 to 100 mg at intervals of 10 to 15 minutes until the desired blood pressure is achieved (Wilson and Vidt, 1978). Diazoxide can also be given by slow intravenous infusion of the undiluted solution at a rate of 15 to 30 mg per minute (Garrett and Kaplan, 1982). Prior administration of a β-adrenergic antagonist will enhance the hypotensive effect of the drug. Diazoxide should not be used to treat hypertension associated with aortic coarctation, arteriovenous shunts, or aortic dissection. Similarly, risks outweigh benefits in its use for intracerebral hemorrhage, acute pulmonary edema, and acute ischemic heart disease.

Toxicity and Precautions. The two most common side effects caused by diazoxide are salt and water retention and hyperglycemia. Retention of fluid can be avoided by restriction of salt and water. The routine use of diuretic agents with diazoxide is not recommended because patients with malignant hypertension are frequently volume depleted. Hyperglycemia results from diazoxide's capacity to inhibit the secretion of insulin from pancreatic β cells. This effect also appears to result from stimulation of ATP-sensitive K^+ channels (Zünkler *et al.*, 1988). The drug does not alter the response to administration of insulin. Thus, hyperglycemia is mainly a problem in non-insulin-dependent diabetic patients who are being treated with oral hypoglycemic agents. Severe hyperglycemia with hyperosmolar, nonketotic coma has been described. Other side effects include tachycardia and myocardial and cerebral ischemia caused by excessive hypotension. Diazoxide relaxes uterine smooth muscle and may arrest labor when used to treat the hypertensive crisis of eclampsia. Rare side effects include gastrointestinal disturbances, flushing, local pain and inflammation after extravasation, altered ability to taste and smell, excessive salivation, and dyspnea. Long-term administration of diazoxide can cause hypertrichosis, as with minoxidil.

Ca^{2+}-CHANNEL BLOCKERS

Ca^{2+}-channel blocking agents are emerging as a very important group of drugs for the treatment of hypertension. The general pharmacology of these drugs is presented in Chapter 32. The antihypertensive effect of Ca^{2+}-channel blockers was demonstrated over 20 years ago, but these drugs have undergone rigorous evaluation for the treatment of hypertension only in the past decade. The logic behind their use for this

purpose comes from the understanding that fixed hypertension is the result of increased peripheral vascular resistance. Since contraction of vascular smooth muscle is dependent on the free intracellular concentration of Ca^{2+}, inhibition of transmembrane movement of Ca^{2+} should decrease the total amount of Ca^{2+} that reaches intracellular sites. Indeed, all of the Ca^{2+}-channel blockers lower blood pressure by relaxing arteriolar smooth muscle and decreasing peripheral vascular resistance (Lehmann *et al.*, 1983). However, unlike other arteriolar dilators, Ca^{2+}-channel blockers do not cause fluid retention, and only the dihydropyridines (nifedipine, nitrendipine, and nicardipine) produce a mild-to-moderate reflex tachycardia (Frishman *et al.*, 1987). Because both verapamil and diltiazem have direct effects on the sinoatrial (SA) node to decrease heart rate, reflex tachycardia is usually not significant with these two drugs. All Ca^{2+}-channel blockers are equally effective when used alone for the treatment of mild-to-moderate hypertension, and in comparative trials, Ca^{2+}-channel blockers are as effective as β-adrenergic antagonists or diuretics (Doyle, 1983; Inouye *et al.*, 1984).

The Ca^{2+}-channel blockers are well tolerated, and only a small fraction of patients discontinue the drug because of an adverse reaction. The dihydropyridines cause the highest incidence of vascular side effects. Approximately 10% of patients develop headache, flushing, dizziness, and peripheral edema. However, edema is clearly not secondary to fluid retention; it most likely results from increased hydrostatic pressure in the lower extremities owing to precapillary dilatation and reflex postcapillary constriction. The most common side effect of verapamil is constipation, while bradycardia occurs most commonly with diltiazem (Russell, 1988).

Although some investigators have advocated the sublingual use of nifedipine for treatment of hypertensive emergencies, it is not readily absorbed from the buccal mucosa; nitroprusside remains the drug of choice because of the ability to titrate the response to the drug quickly. In situations in which close monitoring of blood pressure is not possible, ingestion of 10 to 20 mg of nifedipine can cause a hypotensive effect in 10 minutes, with a maximal effect in 30 to 40 minutes.

Ca^{2+}-channel blockers are versatile drugs with proven efficacy in all types of patients (Kiowski *et al.*, 1986). They seem to be especially efficacious in low-renin hypertension (*i.e.*, blacks and the elderly). The efficacy of Ca^{2+}-channel blockers is enhanced by the concomitant use of a β-adrenergic antagonist, an inhibition of angiotensin converting enzyme, or methyldopa. Diuretics may also enhance the efficacy of Ca^{2+}-channel blockers, but the data have not been consistent. There are significant drug–drug interactions to be recalled when Ca^{2+}-channel blockers are used to treat hypertension. Verapamil can increase plasma concentrations of digoxin (Pedersen *et al.*, 1981). When used with quinidine, Ca^{2+}-channel blockers may cause excessive hypotension, particularly in patients with idiopathic hypertrophic subaortic stenosis. Diltiazem and verapamil must be given with caution to patients who are also receiving a β blocker because of the possible development of AV block or heart failure.

Overall, Ca^{2+}-channel blockers are safe and effective in the treatment of hypertension. They should not be used in patients with SA or AV nodal abnormalities or in patients with overt congestive heart failure. These drugs are usually safe, however, in hypertensive patients with asthma, hyperlipidemia, diabetes mellitus, and renal dysfunction. Unlike β-adrenergic antagonists, Ca^{2+}-channel blockers do not alter exercise tolerance; nor do they alter plasma concentrations of lipids, uric acid, or electrolytes.

ANGIOTENSIN CONVERTING ENZYME INHIBITORS

The importance of the renin–angiotensin system for regulation of cardiovascular function has been appreciated for some time (*see* Chapter 31). The ability to inhibit the activity of the system with orally effective inhibitors of angiotensin converting enzyme is more recent. Captopril was the first such agent to be marketed for the treatment of hypertension. Since then, enalapril

and lisinopril have also become available. These drugs have proven to be very useful for the treatment of hypertension because of their efficacy and their very favorable profile of side effects, which enhances compliance. Although elderly hypertensive patients tend to have lower plasma renin activity than do younger individuals, angiotensin converting enzyme inhibitors have equal antihypertensive efficacy in the two groups (Cooper *et al.*, 1987). Black hypertensive patients are somewhat resistant to the hypotensive effect of these drugs; however, the concurrent use of a diuretic overcomes this relative resistance. These drugs are discussed in detail in Chapter 31.

II. Therapy of Hypertension

NONPHARMACOLOGICAL THERAPY OF HYPERTENSION

Interest in nonpharmacological methods to lower blood pressure arises in part from the fact that about 70% of hypertensive subjects have a mild, asymptomatic elevation of blood pressure. To maintain compliance with a therapeutic regimen, the intervention should not lessen the quality of life. All drugs have side effects. If minor alterations of normal activity or diet can reduce blood pressure to a satisfactory level, the complications of drug therapy can be avoided. In addition, nonpharmacological methods to lower blood pressure allow the patient to participate actively in the management of his or her disease. Reduction of weight, restriction of salt, and moderation in the use of alcohol may reduce blood pressure and improve the efficacy of drug treatment. In addition, regular isotonic exercise, relaxation therapy, and increased consumption of K^+ may also lower blood pressure in hypertensive patients.

Smoking *per se* does not cause hypertension. However, smokers do have a higher incidence of malignant hypertension (Isles *et al.*, 1979), and smoking is a major risk factor for coronary heart disease. Hypertensive patients should stop smoking. Consumption of caffeine can raise blood pressure and elevate plasma concentrations of norepinephrine, but long-term consumption of caffeine causes tolerance to these effects and has not been associated with the development of hypertension. An increased intake of Ca^{2+} has been reported by some investigators to lower blood pressure. The mechanism of this effect is not understood, but suppression of the secretion of parathyroid hormone is apparently involved. However, supplemental Ca^{2+} does not lower blood pressure when populations of hypertensive subjects are studied. Although it is possible that there are some hypertensive patients who have a hypotensive response to Ca^{2+}, there is no easy way to identify such individuals. Supplemental use of Ca^{2+} for this purpose cannot be recommended at the present time (Kaplan, 1988b).

Reduction of Body Weight. Obesity and hypertension are closely associated, and the degree of obesity is positively correlated with the incidence of hypertension. Obese hypertensives may lower their blood pressure by losing weight regardless of a change in salt consumption (Maxwell *et al.*, 1984). The mechanism by which obesity causes hypertension is unclear, but increased secretion of insulin in obesity could result in insulin-mediated enhancement of renal tubular reabsorption of Na^+ and an expansion of extracellular volume. Obesity is also associated with increased activity of the sympathetic nervous system; this is reversed by weight loss. Maintenance of weight loss is difficult for many. A combination of aerobic physical exercise and dietary counseling may enhance compliance.

Sodium Restriction. High salt diets are associated with a high prevalence of hypertension (MacGregor, 1985). Severe restriction of salt will lower the blood pressure in most hospitalized hypertensive patients; this treatment method was advocated prior to the development of effective antihypertensive drugs (Kempner, 1948). However, severe salt restriction is not practical from a standpoint of compliance. Several studies have shown that moderate restriction of salt intake to approximately 5 g per day will, on average, lower blood pressure by 12 mm Hg systolic and 6 mm Hg diastolic. The higher the initial blood pressure, the greater the response. In addition, subjects over 40 years of age are more responsive to the hypotensive effect of moderate restriction of salt (Grobbee and Hofman, 1986). However, not all hypertensive patients respond to restriction of salt. Nonetheless, this intervention is benign and can easily be tried as an initial approach in all patients with mild hypertension. An additional benefit of salt restriction is improved responsiveness to some antihypertensive drugs.

Alcohol Restriction. Consumption of alcohol can raise blood pressure, but it is unclear how much

Selection of Therapy. Until recently, initial therapy for hypertension consisted of either a thiazide diuretic or a β-adrenergic antagonist. However, numerous studies have shown that the Ca^{2+}-channel blockers and inhibitors of angiotensin converting enzyme are also effective as first-line treatment. In choosing initial drug therapy, issues such as side effects, quality of life, cost, and efficacy of drugs in certain subgroups of hypertensive patients should be considered. Thus, the simplified "stepped care approach," previously advocated by most investigators, has been replaced by a more individualized approach in which the patient's age, race, concomitant diseases and therapies, life style, and even, possibly, socioeconomic status are considered.

Thiazide diuretics are the least expensive of the first-line drugs and should be considered for the treatment of hypertension in the elderly and in patients with volume-dependent hypertension. Most black and obese hypertensives belong in the latter group. Thiazides should be avoided in patients with hyperuricemia, hyperglycemia, hyperlipidemia, and hypokalemia. Hypertensive individuals with left ventricular hypertrophy should not be treated with a thiazide diuretic alone, since these drugs have not been shown to reverse the hypertrophy, even though blood pressure is reduced (Drayer *et al.*, 1982).

β-Adrenergic antagonists should be considered for young hypertensive patients and patients with angina pectoris, a history of myocardial infarction, cardiac arrhythmias, or mitral-valve prolapse.

Ca^{2+}-channel blockers should be considered for patients who cannot tolerate diuretics or β-adrenergic antagonists. In general, hypertensive patients with low renin levels respond well to Ca^{2+}-channel blockers. Thus, elderly and black hypertensive patients who have underlying bronchospastic pulmonary disease are good candidates to receive one of these drugs. Patients with left ventricular hypertrophy and/or a history of cardiac arrhythmias and those with peripheral vascular disease can safely be given a Ca^{2+}-channel blocker.

Inhibitors of angiotensin converting enzyme can be used for initial therapy in patients who cannot tolerate diuretics or β blockers. These drugs are also appropriate in patients with congestive heart failure or diabetes mellitus and can be used safely in those with left ventricular hypertrophy and/or cardiac arrhythmias.

Patients who do not respond to a single drug can be switched to another drug with a different mechanism of action. If treatment with a single drug is not successful, addition of a second drug from a different class is appropriate. For example, diuretics will greatly enhance the hypotensive potency of converting enzyme inhibitors. Diuretics also add to the antihypertensive efficacy of β-adrenergic antagonists and Ca^{2+}-channel blockers. If two drugs do not control the blood pressure adequately a third drug of a different class can be added to the existing regimen. However, before proceeding to the next level of therapy, the physician should always consider certain explanations for the inadequate response. Noncompliance must be ruled out carefully, especially if the drug is causing side effects. Drug dosage may be inappropriate or the patient may metabolize the drug rapidly. The concomitant use of drugs that can reverse or alter the effect of antihypertensive agents should be evaluated. Volume overload should always be considered, since excessive retention of fluid can oppose the action of many antihypertensive drugs. Excess intake of alcohol can increase blood pressure, and people who consume alcohol excessively tend to be noncompliant with their therapeutic regimen. Progressive renal insufficiency or surgically correctable causes of hypertension should be evaluated in patients with refractory hypertension.

An attempt to withdraw drug therapy can be made with patients with mild hypertension who have had a satisfactory response to a drug for at least a year, but nonpharmacological means to reduce blood pressure should be continued. Although such patients may at first remain normotensive, the majority will eventually require drug therapy again; careful follow-up is thus of the utmost importance.

The effect of antihypertensive therapy on the quality of life has not been studied frequently, but the fact that more than 10% of patients in large studies stop taking antihypertensive medications because of side ef-

fects cannot be overlooked. Croog and coworkers (1986) compared the effects of captopril, methyldopa, and propranolol on the quality of life of hypertensive patients. Captopril was the least likely to interfere with everyday living in terms of side effects and measures of satisfaction; methyldopa was the least satisfactory, and propranolol fell in between these two. More such studies are needed.

Hypertensive Emergencies. Hypertensive emergencies are situations that require immediate intervention to lower the blood pressure. It is never the absolute blood pressure that defines the emergency, but the damage that is caused by the elevated pressure. Examples of emergencies include hypertensive encephalopathy, intracranial hemorrhage, acute left ventricular failure with pulmonary edema, unstable angina pectoris, acute myocardial infarction, dissecting aortic aneurysm, eclampsia, head trauma, and extensive burns. Patients with these conditions are best treated with parenteral drugs; sodium nitroprusside is most suitable because of the rapidity with which control can be achieved and the level of blood pressure adjusted. For the treatment of aortic dissection, a parenteral β-adrenergic antagonist is necessary prior to administration of nitroprusside. Aortic dissection can also be treated with trimethaphan. Although many hypertensive emergencies can also be treated with small intravenous doses of diazoxide, the drug is contraindicated in aortic dissection and in patients with ischemic cardiovascular disease. In the treatment of hypertensive encephalopathy, it is critical not to lower the blood pressure too quickly because of inadequate autoregulation of cerebral blood flow. It is reasonable to lower blood pressure by 15% quickly and then toward normal over the next 24 hours. Once parenteral antihypertensive therapy is initiated, it is important to begin oral treatment so that the parenteral drug can be discontinued as soon as possible.

Hypertensive urgencies are situations in which the blood pressure should be lowered within several hours, such as in patients with malignant hypertension and progressive renal insufficiency but without signs of encephalopathy. Accelerated administration of oral antihypertensive medications can result in adequate control of blood pressure in such conditions without resorting to parenteral drugs. Since many patients with hypertensive emergencies or malignant hypertension are somewhat volume depleted, the routine use of diuretics should be avoided.

Hypertensive crises in pediatric patients are generally treated with the same drugs that are used in adults, with appropriate modification of dose (Report of the Second Task Force on Blood Pressure Control in Children, 1987). Management of acute hypertension during pregnancy has been reviewed by Maikranz and Lindheimer (1987). Drugs to be avoided in pregnancy include angiotensin converting enzyme inhibitors and sodium nitroprusside.

Treatment of Isolated Systolic Hypertension. Isolated systolic hypertension (systolic blood pressure greater than 160 mm Hg and diastolic blood pressure less than 90 mm Hg) is a common finding in the elderly. When isolated systolic hypertension occurs in a young patient, it is usually indicative of a hyperdynamic circulation, and diastolic hypertension may develop later. An elevation of systolic blood pressure clearly increases the risk of cardiovascular morbidity and mortality in elderly patients. However, there are no definitive data to show that treatment of isolated systolic hypertension will reduce this risk. Definitive recommendations must await the conclusions of an ongoing study, the Systolic Hypertension in the Elderly Program. Until then, the physician should evaluate the risk–benefit ratio for each patient, keeping in mind the fact that the elderly are prone to develop complications from drugs. In the feasibility phase of the Systolic Hypertension in the Elderly Program, chlorthalidone was found to be effective for treatment of systolic hypertension with an acceptable incidence of side effects (Hulley *et al.*, 1985); Ca^{2+}-channel blockers are reasonable alternatives.

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The quantitative determination of several inhibitors of the angiotensin-converting enzyme by CE

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Abstract

Capillary electrophoresis (CE) was applied to the study of several inhibitors of the angiotensin-converting enzyme. Separation of the compounds was performed by means of two phosphate buffers (each 100 mM) at pH 7.0 and 6.25, respectively [S. Hillaert, W. Van den Bossche, J. Chromatogr. A, 895 (2000) 33–42.]. Due to the highest selectivity of the first mentioned running buffer, the same system has been applied for the quantification of enalapril, lisinopril, quinapril, fosinopril, perindopril and benazepril in their corresponding pharmaceutical formulation. Especially, the possibility of simultaneous identification and quantification of the active ingredient in the finished product is very attractive. Excipients do not adversely affect the results. This paper deals with the validation of some parameters of the quantitative analysis: linearity, precision, accuracy and robustness. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Capillary electrophoresis; ACE inhibitors; Tablets; Quantitative analysis

1. Introduction

The inhibitors of the angiotensin-converting enzyme (ACE inhibitors) are widely used for the treatment of mild to moderate hypertension and heart failure, either alone or in conjunction with other drugs [2]. The first developed ACE inhibitor was captopril, a thiol-containing compound. Since captopril causes some side effects and researchers believed that the thiol group was responsible for these side effects, it was preferable to develop non-thiol-containing ACE inhibitors [3].

There are three classes of new ACE inhibitors, according to the group that enhances the binding to the zinc ion of the angiontensin-converting enzyme. The first class has a second carboxyl group and lisinopril and enalaprilat (the active metabolite of enalapril maleate that normally is used as drug) are the only representatives. Fosinopril, a phosphorus-containing ACE inhibitor, forms part of the second class. It is inactive but serves as a prodrug, being completely hydrolyzed to the active diacid, fosinoprilate. The third class or all the other ACE inhibitors, viz., enalapril

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maleate, quinapril, perindopril, and benazepril possess a carboxylic acid ethyl ester and have the common property of acting as prodrugs, being converted to the active diacid by metabolism by liver and intestinal enzymes (third class) [2,3].

Until now, high performance liquid chromatography (HPLC) has been a major technique used for the quantitative determination of the ACE inhibitors [4–19]. The same technique was also applied in the monograph about enalapril maleate, lisinopril dihydrate and ramipril in the European Pharmacopoeia [20].

Analysis by means of capillary electrophoresis (CE) has been achieved for the identification of eight ACE inhibitors [1]. Other studies have been limited to the determination and rotamer separation of enalapril maleate [21–23] and lisinopril [24]. One study has reported on the determination of fosinopril and its related impurities [25]. Another study has been limited to the determination of only four ACE inhibitors [26] while our study has investigated the separation of eight ACE inhibitors [1].

The aim of this study was to investigate if the method, able to separate a large number of ACE inhibitors, could also be used for the quantification of these compounds [1]. The system is appropriate for quantitative determination in different pharmaceutical formulations without specific sample pretreatment.

This paper deals with the validation of the most important parameters for the quantitative analysis.

Table 1
Selection of the internal standard

Substance to be examined	Appropriate internal standard
Enalapril	Lisinopril
	Fosinopril
	Cilazapril
	Ramipril
	Quinapril
Lisinopril	All the other ACE inhibitors
Quinapril	Lisinopril
	Fosinopril
	Enalapril
Fosinopril	Lisinopril
	Quinapril
	Ramipril
	Benazepril
	Enalapril
Perindopril	Lisinopril
	Fosinopril
Benazepril	Lisinopril
	Fosinopril

Table 2
Reference solutions for the quantitative determination

Reference substance	Reference solution (mg/50 ml)	Diluted reference solution (mg/ml)
Enalapril maleate	±175	±1.87
Lisinopril dihydrate	±250	±2.67
Quinapril · HCl	±60	±0.64
Fosinopril · sodium	±150	±1.60
Perindopril *t*-butylamine	±125	±1.33
Benazepril · HCl	±60	±0.64

2. Experimental

2.1. Instruments

The validation of the method and the experiments were performed on a Crystal CE, equipped with PC 1000 software installed on a IBM computer with OS/2 as the operating system. The capillary used was a fused-silica capillary 60 cm in total length (33 cm to the detector) and 50 μm internal diameter (I.D.). The Crystal CE can be controlled over a large temperature range and the temperature used was 25°C for the tray and 30°C for the capillary.

The sample solutions were introduced into the capillary by pressure injection (50 mbar) for 5 s. A constant voltage of 30 kV was applied and UV absorbance at 214 nm was employed for detection. The detection was by means of a variable-wavelength UV detector (Spectra FOCUS detector).

To demonstrate the ruggedness of the system, some of the work was also performed on a Waters

Table 3
Sample preparation for the quantitative determination

	Average mass (mg)	Sample solution (mg powder/15 ml)	Internal standard solution (mg/ml)	Diluted sample solution (mg active substance/ml)
Enalapril [Renitec®] 20 mg — tablets	203.9	±254 mg	Lisinopril · $2H_2O$: 5 mg	±1.66
Lisinopril [Zestril®] 20 mg — tablets	226.6	±400 mg	Enalapril maleate: 5 mg	±2.35
Quinapril [Accupril®] 20 mg — tablets	208.0	±83 mg	Lisinopril · $2H_2O$: 5 mg	±0.53
Fosinopril [Fosinil®] 20 mg — tablets	201.2	±200 mg	Quinapril · HCl: 2.5 mg	±1.33
Perindopril [Coversyl®] 4 mg — tablets	90	±270 mg	Lisinopril · $2H_2O$: 5 mg	±0.80
Benazepril [Cibacen®] 10 mg — tablets	186.7	±150 mg	Lisinopril · $2H_2O$: 5 mg	±0.54

Quanta 4000 (Millipore, Waters), equipped with a fused-silica capillary 60 cm in total length (52.5 cm to the detector) and 50 μm I.D. The data were collected on a Hewlett-Packard Integrator (HP 3396 Series II), processing both the areas and the heights of the peaks.

The sample solutions were introduced into the capillary by hydrodynamic introduction for 10 s. Hydrodynamic injections were performed by lifting the sample vial approximately 10 cm above the height of the buffer vial for 10 s. A constant voltage of 25 kV was applied and UV absorbance at 214 nm was used for detection, which was by means of an on-line fixed-wavelength UV detector with a zinc discharge lamp and a 214-nm filter.

2.2. Reagents

Sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate dihydrate were obtained from E. Merck (Germany). Enalapril maleate was purchased from Sigma (St. Louis, MO, USA). Lisinopril dihydrate was obtained from Zeneca, quinapril · HCl from Parke-Davis, fosinopril sodium from Bristol-Myers Squibb, perindopril *t*-butylamine from Servier and benazepril · HCl from Ciba-Geigy.

Commercially available drugs [Renitec® 20 mg (MSD), Zestril® 20 (Zeneca), Accupril® 20 mg (Parke Davis), Fosinil® (Solvay), Coversyl® (Servier) and Cibacen® (Novartis)] were used for the quantitative determination.

2.3. Running buffer

The sodium phosphate buffer (pH 7.0; 100 mM) was used as running buffer. It was prepared by adjusting the pH of a 100 mM disodium hydrogen phosphate solution to pH 7.0 by the addition of a 100 mM sodium dihydrogen phosphate solution.

2.4. Internal standard solutions

Selection of the internal standard had to be made on the basis of the substance to be examined (Table 1). Lisinopril dihydrate was chosen mostly as the internal standard because of its baseline separation with all the other ACE inhibitors and because of its availability as bulk product on the market. For the determination of lisinopril, each other ACE inhibitor can be used. An appropriate amount of the compound (Table 3) was dissolved in 20 ml running buffer and diluted to 50 ml with the same running buffer.

2.5. Reference solutions

Reference solutions were prepared by accurately weighing an appropriate amount of the corresponding reference substance, dissolving in

20 ml running buffer and diluting to 50.0 ml with the same buffer solution (Table 2). A volume of 8.0 ml of these solutions was mixed with 5.0 ml of the internal standard solution and diluted to 15 ml with the buffer solution.

2.6. Sample preparations

Minimum twenty tablets were weighed, ground, and mixed. An appropriate amount of the powder (Table 3) was mixed with 5.0 ml of the appropri-

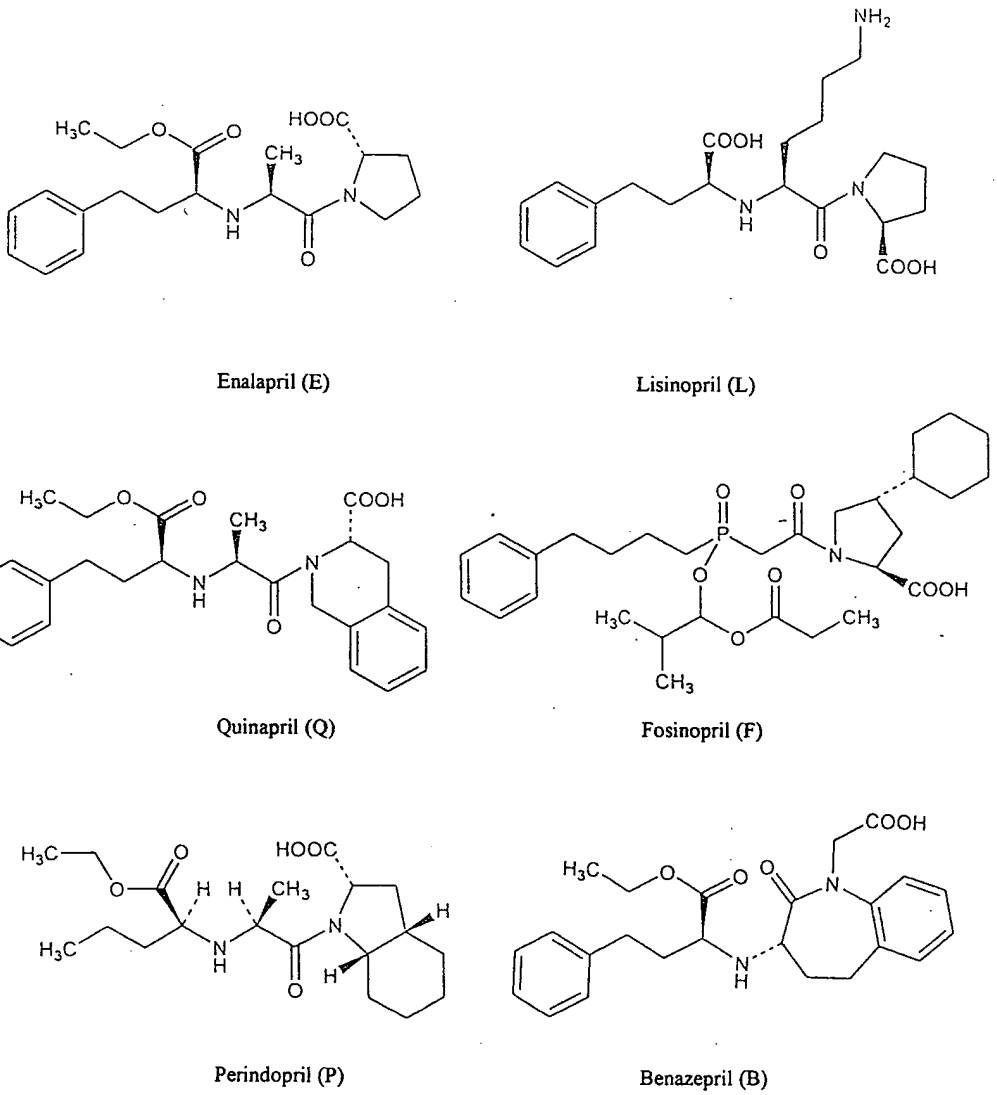

Fig. 1. Chemical structures of the ACE-inhibitors.

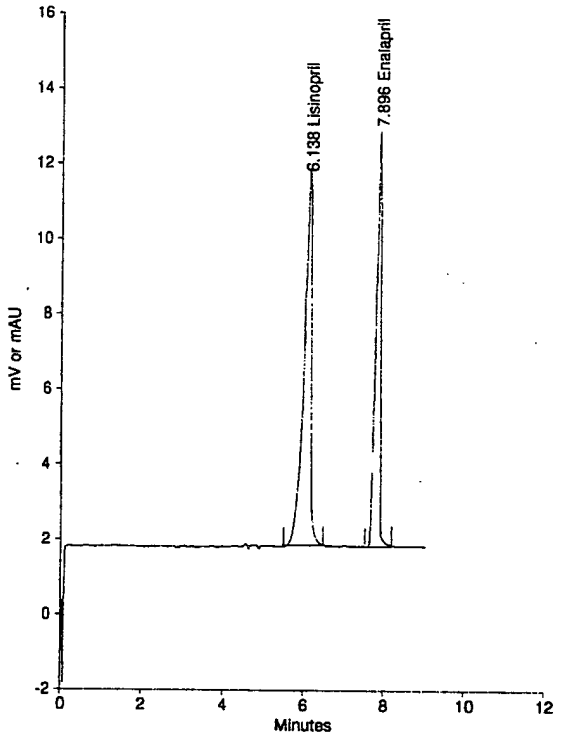

Fig. 2. Electropherogram of the quantitative determination of lisinopril [Zestril®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

ate internal standard solution (Table 3) and diluted to 15 ml with the running buffer.

All the samples and buffers were filtered through a Millipore 0.45 μm filter unit.

3. Results and discussion

3.1. Optimization of the method

Until now, the literature shows no selective method, which is able to separate and quantify several ACE inhibitors. The published studies can only be applied for the quantitative determination of one or two of these compounds [21–26].

The optimization of a selective CE separation of several ACE inhibitors was published earlier [1]. The aim of this study was to investigate if that method could also be used for the quantification of these compounds. Separation was performed by means of two phosphate buffers (each 100 mM) at pH 7.0 and 6.25, respectively. This combination is necessary for the selective identification of the structurally related substances because of their similar pK_a-values [1]. Due to the highest selectivity of the sodium phosphate buffer (pH 7.0; 100 mM) and the good peak shapes, this system has been applied for the quantification of enalapril, lisinopril, quinapril, fosinopril, perindopril and benazepril in their corresponding formulations. The selection of the internal standard had to be made on the basis of the substance to be examined. Due to the specificity of the developed method, the possibility of simultaneous identification and quantification of the active ingredient in the finished product is very attractive.

The chemical structures of the examined ACE inhibitors are represented in Fig. 1.

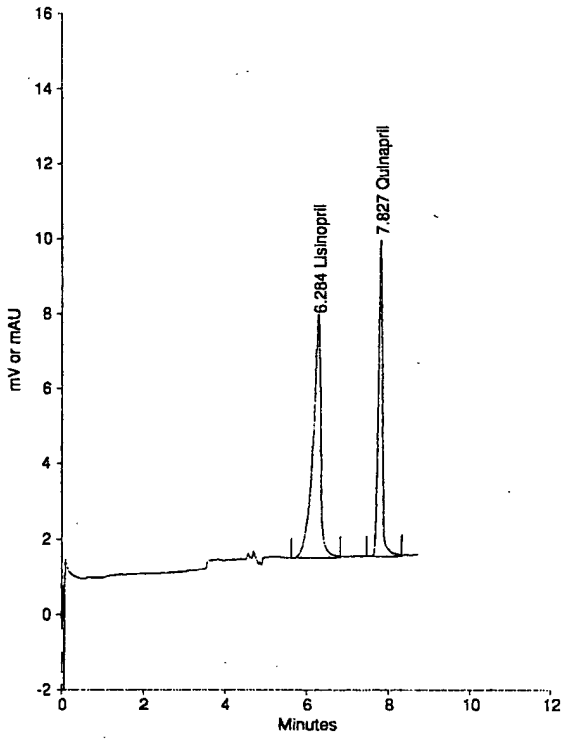

Fig. 3. Electropherogram of the quantitative determination of quinapril [Accupril®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

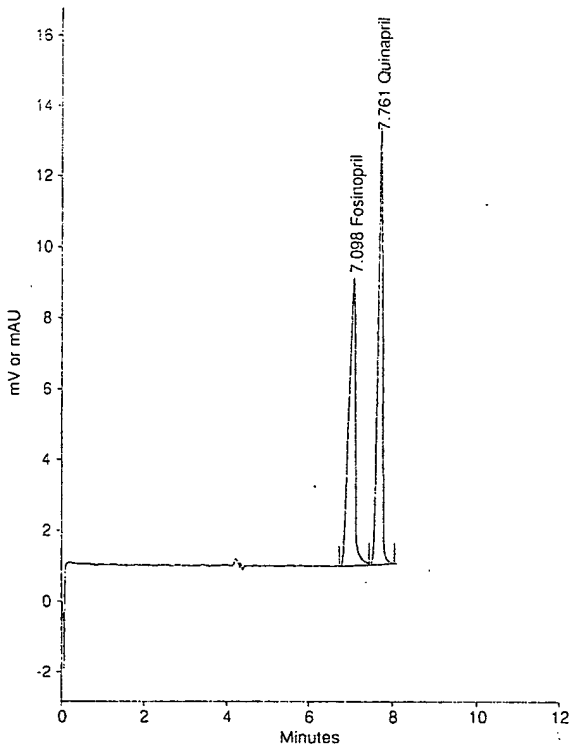

Fig. 4. Electropherogram of the quantitative determination of fosinopril [Fosinil®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

3.2. Quantitative determination in pharmaceutical formulations

A sodium phosphate buffer (pH 7.0; 100 mM) is appropriate for the quantitative determination of the ACE inhibitors (Figs. 2–6). By the means of different placebo mixtures it was demonstrated that the following excipients do not adversely affect the results, lactose, sodium hydrogen car bonate, maize starch, pregelatinized maize starch, mannitol, calcium hydrogen phosphate, magnesium carbonate, gelatin, polyvidone and crospovidone, microcrystalline cellulose, macrogol 400 and 8000, magnesium stearate, silicon dioxide, hypromellose and titanium dioxide.

3.3. Validation of the method

3.3.1. Linearity

The detector responses were found to be linear for the different components in two concentration ranges as mentioned in Table 4. The amount of the internal standard was adapted according to the used concentration range. The regression analysis data for the calibration curves were calculated using the peak areas.

3.3.2. Precision

The precision (repeatability) was determined by the total analysis of six replicate samples under the same operating conditions, by the same analyst, and on the same day. The mean value of the concentration and the relative standard deviation (R.S.D.) are summarized in Table 5.

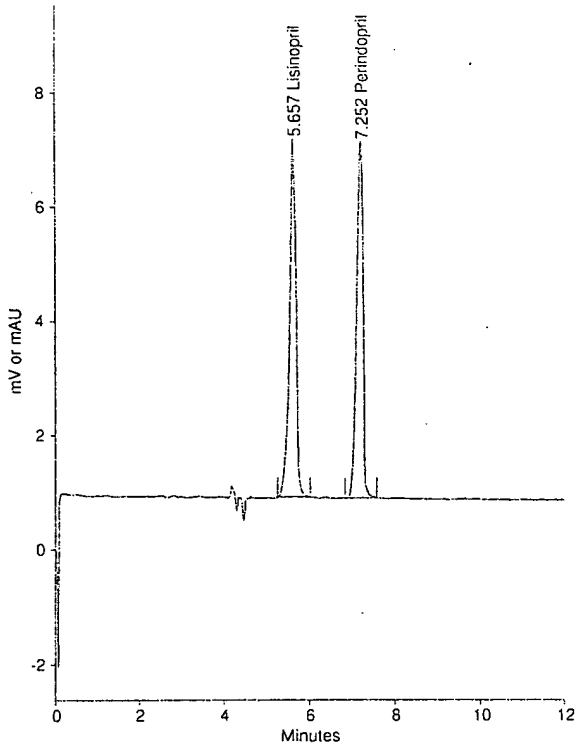

Fig. 5. Electropherogram of the quantitative determination of perindopril [Coversyl®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

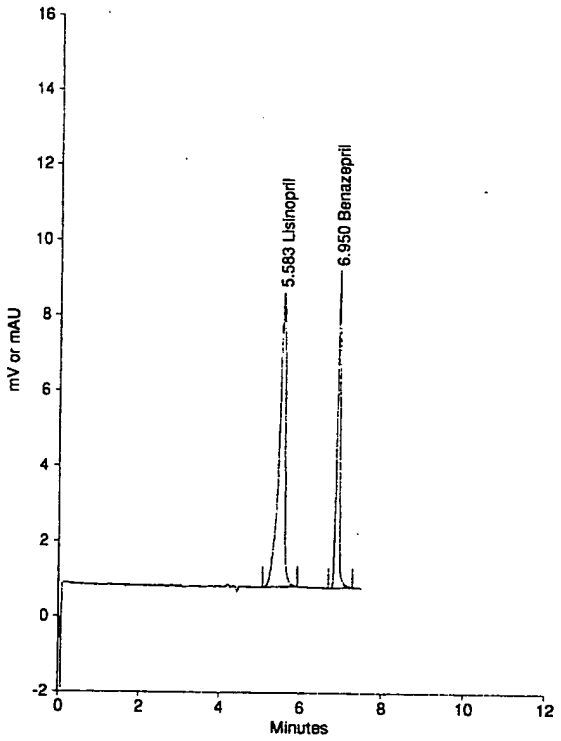

Fig. 6. Electropherogram of the quantitative determination of benazepril [Cibacen®] on a fused-silica capillary, performed on the Crystal CE. Conditions: 60 cm (33 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 30 kV; detection at 214 nm.

Table 4
Linearity

	Concentration range (mg/ml)	Correlation coefficient (r^2)
Enalapril maleate	0.02–0.47	0.9999
	0.47–2.35	0.9999
Lisinopril dihydrate	0.03–0.67	0.9999
	0.67–3.35	0.9999
Quinapril · HCl	0.01–0.20	0.9999
	0.16–0.80	0.9994
Fosinopril sodium	0.02–0.36	0.9999
	0.40–2.00	0.9993
Perindopril *t*-butylamine	0.02–0.33	0.9996
	0.33–1.67	0.9998
Benazepril · HCl	0.01–0.20	0.9999
	0.16–0.80	0.9994

The error of the equipment, the electrophoretic separation, and the relative standard deviation were determined by performing ten consecutive injections of the same sample (Table 6). It was performed on the Waters Quanta 4000.

Table 5
Precision (repetability) of the total analysis of the six replicate samples

Substance to be examined	Theoretical amount (mg/tablet)	Amount found	Relative standard deviation ($n = 6$)
Enalapril maleate [Renitec®]	20 mg	19.68 mg ± 0.02 mg or 98.4%	0.12%
Lisinopril · $2H_2O$ [Zestril®]	20 mg	20.42 mg ± 0.05 mg or 102.1%	0.24%
Quinapril · HCl [Accupril®]	20 mg	20.23 mg ± 0.08 mg or 101.2%	0.39%
Fosinopril sodium [Fosinil®]	20 mg	19.83 mg ± 0.11 mg or 99.2%	0.55%
Perindopril *t*-butylamine [Coversyl®]	4 mg	3.95 mg ± 0.01 mg or 98.8%	0.25%
Benazepril · HCl [Cibacen®]	10 mg	10.20 mg ± 0.02 mg or 102.0%	0.20%

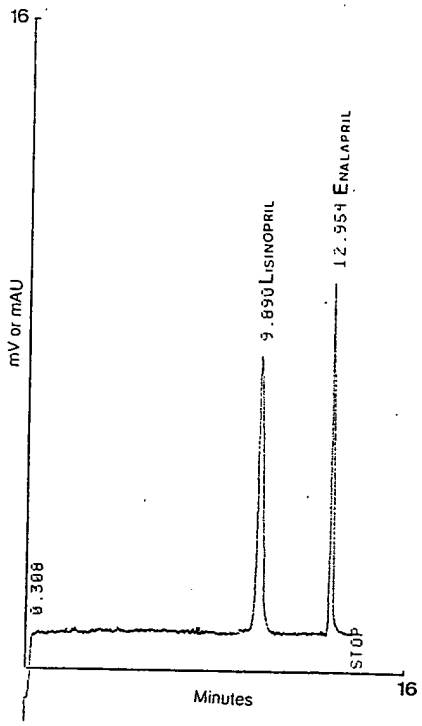

Fig. 7. Electropherogram of the quantitative determination of lisinopril [Zestril®] on a fused-silica capillary, performed on the Waters Quanta 4000. Conditions: 60 cm (52.5 cm to the detector) × 50 μm I.D.; sodium phosphate buffer (pH 7.0; 100 mM) as running buffer; applied voltage, 25 kV; detection at 214 nm.

Table 6
Repetability of ten consecutive injections of the same sample (performed on the Waters Quanta)

Sample solution	Relative standard deviation ($n = 10$)
Enalapril maleate	0.70%
Lisinopril · $2H_2O$	1.09%
Quinapril · HCl	0.68%
Fosinopril sodium	0.53%
Perindopril *t*-butylamine	0.27%
Benazepril · HCl	0.38%

3.3.3. Accuracy

The accuracy of the method was determined by investigating the recovery of each component at three levels ranging from 80 to 120% of the theoretical concentration from placebo mixtures spiked with the active substance (Table 7).

3.3.4. Robustness

To demonstrate the system robustness, the quantitative determination of enalapril, lisinopril, perindopril and benazepril was also performed on a Waters Quanta 4000, equipped with a fused-silica capillary 60 cm in total length (52.5 cm to the

Table 7
Accuracy

	Recovery placebo + 80% ($n = 3$)	Recovery placebo + 100% ($n = 3$)	Recovery placebo + 120% ($n = 3$)
Enalapril	99.6 ± 0.1%	100.6 ± 0.1%	99.2 ± 0.4%
Lisinopril	102.4 ± 0.2%	100.8 ± 0.3%	100.2 ± 0.2%
Quinapril	102.4 ± 0.1%	102.0 ± 0.2%	102.0 ± 0.2%
Fosinopril	9.9 ± 0.3%	100.5 ± 0.2%	100.4 ± 0.1%
Perindopril	100.5 ± 0.2%	100.5 ± 0.4%	100.0 ± 0.2%
Benazepril	101.0 ± 0.2%	100.6 ± 0.3%	100.1 ± 0.1%

Table 8
Robustness

	Waters Quanta 4000		Crystal CE	
	Amount found (mg/tablet)	RSD ($n = 6$)	Amount found (mg /tablet)	RSD ($n = 6$)
Enalapril [Renitec®]	19.63 ± 0.09 mg or 98.2%	0.46%	19.68 ± 0.02 mg or 98.4%	0.12%
Lisinopril [Zestril®]	20.41 ± 0.06 mg or 102.1%	0.29%	20.42 ± 0.05 mg or 102.1%	0.24%
Perindopril [Coversyl®]	3.88 ± 0.03 mg or 97.0%	0.69%	3.95 ± 0.01 mg or 98.8%	0.25%
Benazepril [Cibacen®]	10.16 ± 0.06 mg or 101.6%	0.60%	10.20 ± 0.02 mg or 102.0%	0.20%

detector) and 50 μm I.D. The method conditions with the exception of the running voltage, applied on the Crystal CE could be transferred to the Waters Quanta (Figs. 2 and 7). The results of the quantitative determinations were similar to those on the Crystal CE (Table 8). The R.S.D. of the results on the Waters Quanta 4000 was higher as a result of the temperature fluctuations.

4. Conclusion

The determination of different ACE inhibitors by capillary electrophoresis has been achieved. The study demonstrates that CE can be successfully applied to the quantitative analysis of these compounds in pharmaceutical formulations.

Acknowledgements

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This is Exhibit 4 referred to in the Declaration of Michael M. Lipp, sworn this 9th day of February, 2005.

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Solid-State Chemistry of Drugs

SECOND EDITION

Stephen R. Byrn
Ralph R. Pfeiffer
Joseph G. Stowell

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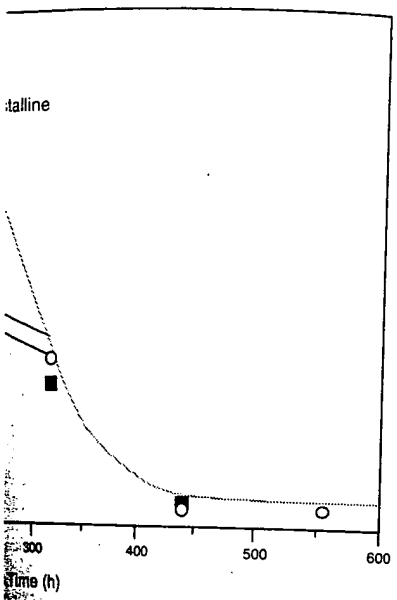
Cover illustration: The figures are space-filling representations of prednisolone 21-*tert*-butylacetate crystal packing diagrams. On the top is Form IV illustrating the densely packed crystal lattice. On the bottom is Form V showing the oxygen-accessible tunnels produced by desolvation.

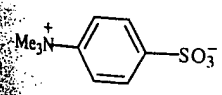
talline



for tetraglycine methyl ester (freeze-dried, crystal-
ed) at 100 °C (Shalaev *et al.*, 1997a).

olysis. These differences among structur-
ors that have not been elucidated.

ULFONATE



4-(trimethylamino)-
benzenesulfonate

hyl-transfer reaction of methyl
trimethylamino)benzenesulfonate
action was studied via a labeling
the ester and the ester-d_6. The
ed that the reaction is $\geq 76\%$

ealed that it was 25–40 times
indicates that the reaction is
on were done at only 15 °C
the reactant ester provided
e 20.9 shows a view of the

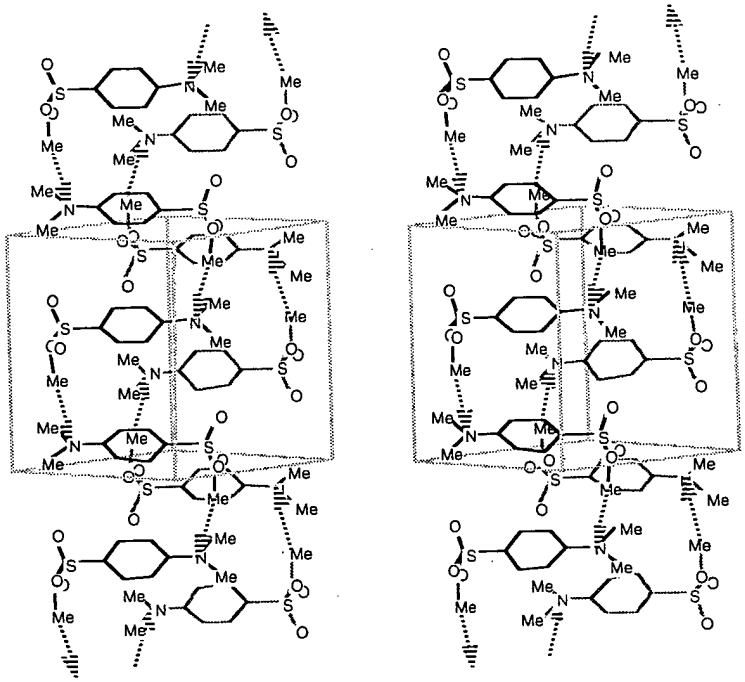


Figure 20.9 Stereoview of the stacking of one chain of methyl 4-(dimethylamino)benzenesulfonate molecules viewed perpendicular to the (101) plane. The hatched arrows shows the methyl groups that is transferred from the $—SO_3$ groups to the $Me_2N—$ groups during the reaction (Sukenik *et al.*, 1977).

It is obvious from Figure 20.9 that very little molecular movement is required for the reaction to occur, and in fact the molecules are in an almost perfect orientation for reaction. Indeed, the solid-state reaction is 25–50-fold faster than the liquid reaction.

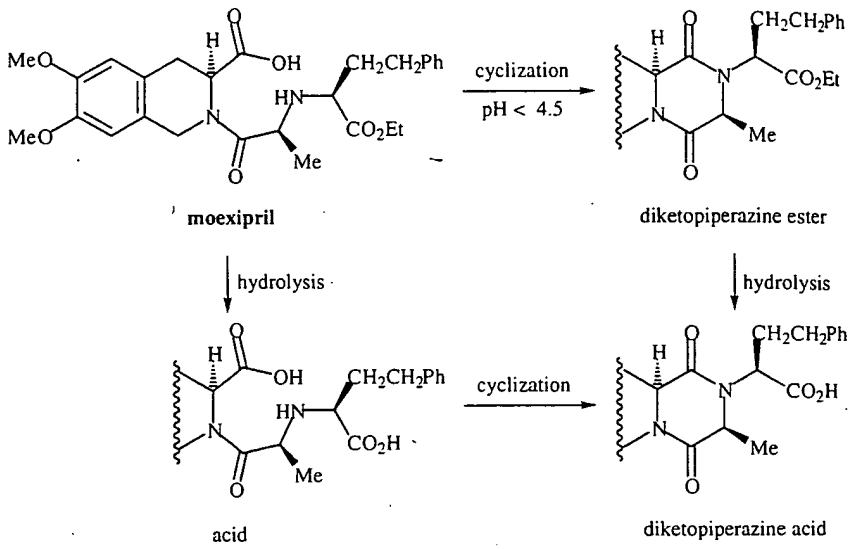
A study by Menger and coworkers (1984) showed that the rate of the methyl transfer in the sulfonate ester was slower at the surface than in the bulk of the solid. This result further establishes that the favorable orientation of the reacting groups accelerates the reaction. The data on this sulfonate ester make it tempting to speculate that a similar type of crystal packing favors the methyl transfer in the tetraglycine methyl ester. Such packing would also emphasize that further experiments aimed at explaining the reaction of tetraglycine methyl ester are definitely in order.

D. ACE Inhibitors

Angiotensin-converting enzyme (ACE) inhibitors such as moexipril degrade in the solid state, especially in the presence of excipients (Strickley *et al.*, 1989; Gu *et al.*, 1990). Analysis of the degradation of moexipril in solution and the solid state showed that the acid, the diketopiperazine ester, and the diketopiperazine acid were the main products.

The stability of moexipril in the presence of various excipients in the dry state and in the presence of 15% water was studied. In the presence of moisture, degradation occurred in all mixtures but the basic excipients stabilized moexipril more than the

acidic excipients. Basic excipients favored direct cyclization over hydrolysis in the presence or absence of water whereas the moexipril is relatively stable in the presence of acidic excipients in the dry state. This is consistent with solution stability studies where bases are more effective at stabilizing moexipril than acids. In wet granulations, the same order of stability is observed as that in solution. These data suggest that the mechanism of the solid-state reaction and the solution reaction may be different; however more research needs to be done to verify this hypothesis.



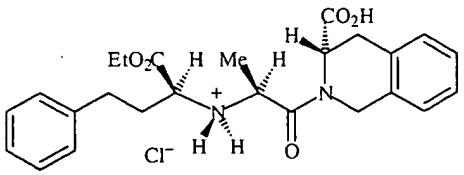
moexipril

diketopiperazine ester

acid

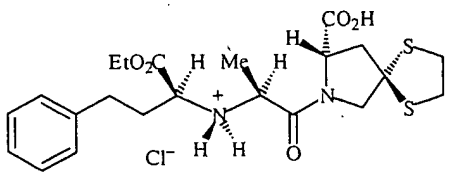
diketopiperazine acid

Xu (1997) has conducted a study of the solid-state reactivity of two model ACE inhibitors: spirapril hydrochloride and quinapril hydrochloride. Both of these compound cyclize to form diketopiperazines as shown in Figure 20.10. This cyclization requires deprotonation of the reacting amine followed by addition of the neutral nitrogen to the carbonyl of the neighboring carboxylic acid to form a tetrahedral intermediate. This intermediate then loses water to give the diketopiperazine product. The



quinapril hydrochloride

[8*S*-[7[*R(*R**)],8*R**]]-7-[2[[1-(ethoxycarbonyl)-3-phenylpropyl]-amino]-1-oxopropyl]-1,4-dithia-7-azaspiro[4,4]nonane-8-carboxylic acid hydrochloride**



spirapril hydrochloride

[3*S*-[2[*R(*R**)],3*R**]]-2-[2-[[(1-ethoxycarbonyl)-3-phenylpropyl]-amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquionoline carboxylic acid hydrochloride**

: cyclization over hydrolysis in the
il is relatively stable in the presence
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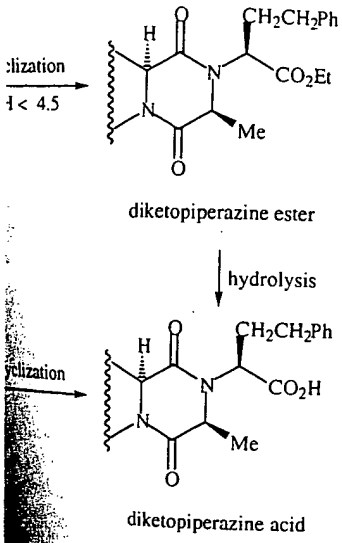


te reactivity of two model ACE
chloride. Both of these com-
igure 20.10. This cyclization
addition of the neutral nitro-
form a tetrahedral intermedi-
etopiperazine product. The

april hydrochloride

R*)],8R*]]-7-[2[[1-(ethoxy-
henylpropyl]-amino]-1-oxo-
inia-7-azaspiro[4,4]nonane-
ic acid hydrochloride

hydrochloride

]-2-[2-[[(1-ethoxy-
opyl]-amino]-1-oxo-
ydro-3-isoquionoline
hydrochloride

crystal structure of quinapril hydrochloride acetonitrile solvate (Hausin and Codding, 1991) and spirapril hydrochloride monohydrate (Xu *et al.*, 1997) have been determined and the reacting atoms (the nitrogen of the alanine residue and the carbonyl of the neighboring carboxylic acid) are greater than 5 Å apart (Xu, 1997). Thus, a conformational change must occur in this portion of the molecule for the reaction to take place in the solid state.

The reaction of crystalline and amorphous spirapirl hydrochloride was studied at 75, 80, 90, 95, and 100 °C over Drierite® (RH ~0%) (see Figure 20.11). The formation of the diketopiperazine was monitored using HPLC and the physical stability was monitored using XRPD. Plots of the solid-state concentration of diketopiperazine

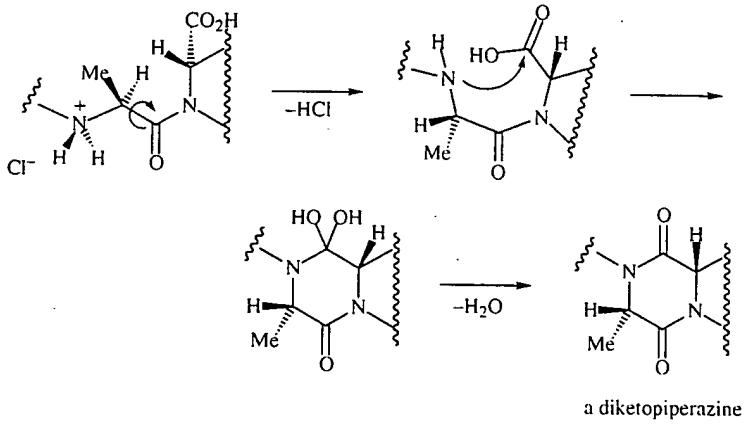


Figure 20.10 Mechanism scheme for the cyclization of an ACE inhibitor to the corresponding diketopiperazine product (Xu, 1997).

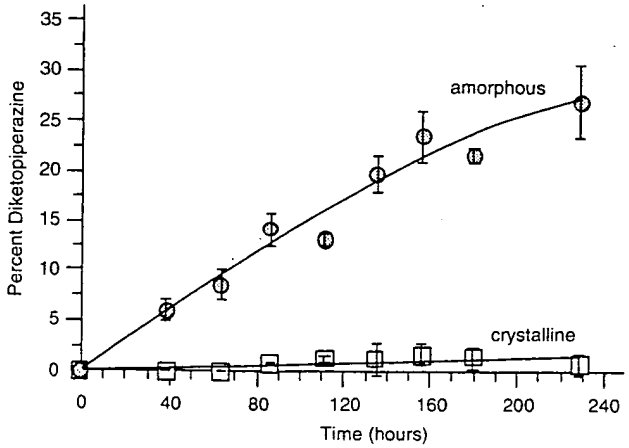


Figure 20.11 Rates of degradation of crystalline and amorphous spirapril hydrochloride at 75 °C (Xu, 1997).

versus time were fitted to a zero-order equation and the rate constant was determined. Crystallization of amorphous spirapril hydrochloride was faster than diketopiperazine formation at 100 °C, thus no rate constant could be determined for this temperature. This data shows that crystalline spirapril hydrochloride is much more stable than the amorphous form. This is consistent with other comparisons of the rates of reactions of crystalline and amorphous materials and the generally held concept that the mobility of crystalline materials is much less than that of amorphous materials. This observation is confirmed by the interrupted-decoupled solid-state NMR spectra which show that amorphous spirapril hydrochloride had much more mobility than the crystalline form.

Quinapril hydrochloride crystallized as an organic solvate and an unsolvated crystal form has not been reported. The acetonitrile solvate desolvates to produces amorphous quinapril hydrochloride. For this reason, it was impossible to directly compare the rate of reaction of a crystalline sample with that of an amorphous sample. Nevertheless, the rate of degradation of amorphous quinapril hydrochloride is faster than that of the acetonitrile solvate even though the solvate is desolating to the amorphous form during the reaction (Xu, 1997). These studies show that as mobility is increased (by forming the amorphous form) the rate of degradation increases.

Xu (1997) also investigated the effect of water on the reactivity of amorphous quinapril hydrochloride and spirapril hydrochloride. Water, which has a very low glass-transition temperature T_g, significantly lowers the T_g of both quinapril hydrochloride and spirapril hydrochloride when present in these amorphous compounds as shown in Figure 20.12. This decrease in T_g correlates with an increase in reactivity (see Figure 20.13).

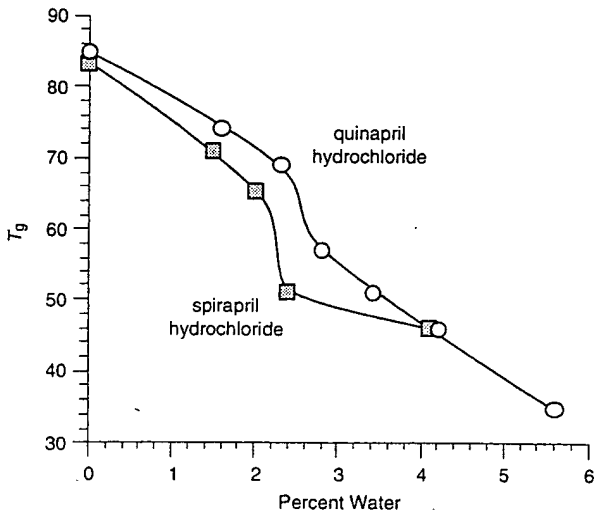


Figure 20.12 The effect of water content on the T_g of quinapril hydrochloride and spirapril hydrochloride (Xu, 1997).

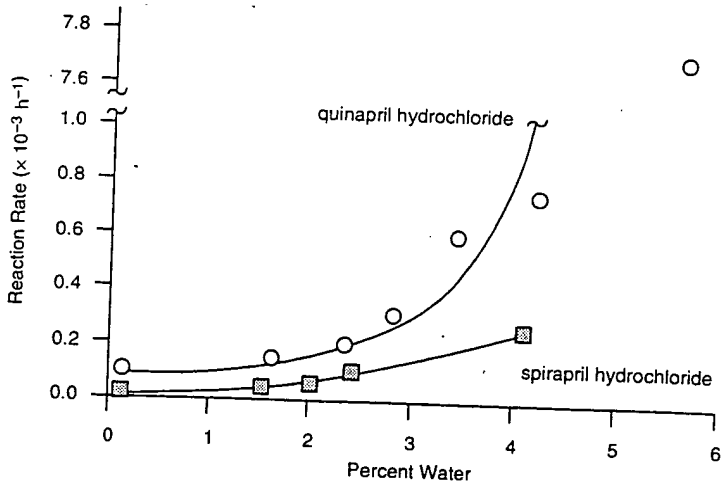


Figure 20.13 Effect of water content on the reaction rate of amorphous spirapril hydrochloride and quinapril hydrochloride (Xu, 1997).

Solid state ^{13}C CP/MAS NMR was used to study the mobility of several of the carbon atoms in amorphous quinapril hydrochloride. The T_1 relaxation time of carbon C21 was particularly noteworthy (see Figure 20.14). At temperatures well below the T_g (low moisture contents), the T_1 values of C21 are much less temperature dependent than the corresponding T_1 values near and above T_g (compare the behavior of T_1 values at high and low relative humidities). Independent studies show that the lower the T_1 relaxation time, the higher the mobility. Thus, the changes of the T_1 relaxation time with both temperature and relative humidity indicate that C21 has greater mobility at higher temperatures and higher relative humidities.

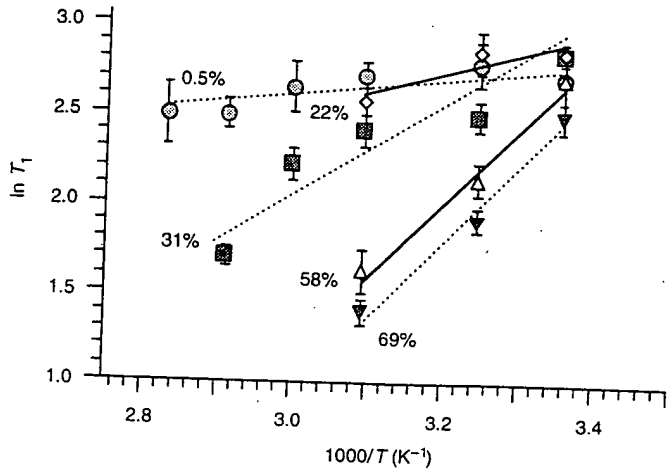


Figure 20.14 Temperature and relative humidity dependence of the T_1 relaxation time of carbon C21 in amorphous quinapril hydrochloride (Xu, 1997).

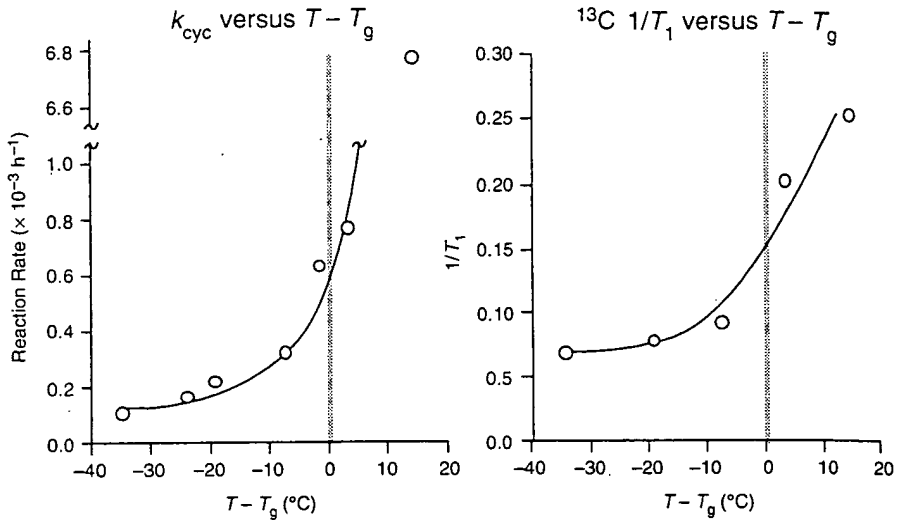
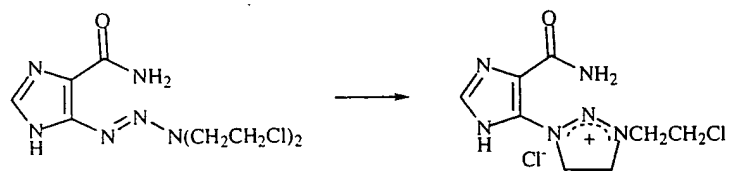


Figure 20.15 Plots of the rate of cyclization to diketopiperazine versus T–T_g and T_1 versus T–T_g for amorphous quinapril hydrochloride (Xu, 1997).

It is of interest to replot the data in Figures 20.13 and 20.14 using an x-axis of T–T_g since this term represents the temperature above or below T_g (see Figure 20.15). Figure 20.15 shows that as T_g is approached both the mobility and reactivity increase dramatically.

In conclusion, this study shows that mobility is related to reactivity in this important series of drugs. In addition, it shows that amorphous materials must be kept at temperatures significantly below their T_g to prevent instability (extrapolations show that the rate constant for these reactions drops to nearly zero at about 50 °C below T_g). The relationship between mobility and reactivity will be discussed in more detail in Chapter 22.

E. Triazenoimidazole



5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide

5-[3-(2-chloroethyl)-1*H*-triazolinyl]-imidazole-4-carboxamide chloride

A potentially useful antileukemic agent, 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide, was reported to rapidly isomerize in the solid state to form an ionic chloride (Shealy and Krauth 1966; Shealy *et al.*, 1968; James *et al.*, 1969). Subsequent single-crystal X-ray studies showed that the ionic chloride was a triazolinium

This is Exhibit 5 referred to in the Declaration of Michael M. Lipp, sworn this 9th day of February, 2005.

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Pharmaceutical Research, Vol. 10, No. 6, 1993

Research Article

Mechanism and Kinetics of Metal Ion-Mediated Degradation of Fosinopril Sodium

Ajit B. Thakur,[1,3] Kenneth Morris,[1] John A. Grosso,[1] Kenneth Himes,[1] John K. Thottathil,[1] Robert L. Jerzewski,[1] Deodatt A. Wadke,[1] and J. Thurø Carstensen[2]

Received July 21, 1992; accepted December 15, 1992

Fosinopril sodium (I), a new angiotensin converting enzyme inhibitor, is a diester prodrug of the active moiety II. We report here a novel transformation of fosinopril into β-ketoamide, III, and a phosphonic acid, IV, mediated through metal ion participation. The interaction of fosinopril with magnesium ions was studied in a solution model system in which methanol was used as the solvent and magnesium acetate as the source of metal ions. Kinetic analysis indicated the degradation to be a bimolecular process, with the rate being first order in both metal ion and fosinopril concentration. The degradation products II, III, and IV effectively retarded the magnesium ion mediated reaction of fosinopril. Based on the results of ^{31}P-NMR, ^{1}H-NMR, Mn(II)-EPR spectroscopy experiments and mass spectrometry, a mechanism is postulated for this transformation. A key reactive intermediate has been characterized that supports the proposed mechanism. The results can account for the observed degradation profile of the fosinopril sodium in a prototype tablet formulation.

KEY WORDS: fosinopril sodium; magnesium ions; C–P bond cleavage; kinetics; mechanism; tablet formulation.

INTRODUCTION

Fosinopril, [1[*S**(*R**)]2α,4β]-4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)-propoxy](4-phenylbutyl)phosphinyl]-acetyl]-L-proline, sodium salt (I) (Scheme I), is a new angiotensin converting enzyme inhibitor marketed under the trade name Monopril® (1). Fosinopril has four chiral centers and theoretically should exist in 16 isomeric forms. However, its synthesis is designed to give 99.9% S R S S isomer. It is a prodrug which is converted *in vivo* into the active moiety II by the hydrolysis of the diester side chain (1). In this communication we report a novel metal ion mediated rearrangement that results in degradation of fosinopril into a β-ketoamide, III, and a phosphonic acid, IV. The degradation product III was isolated from the tablets undergoing accelerated stability testing and was characterized by ^{1}H NMR and MS. Its structure was confirmed by unambiguous synthesis. Compound IV is reported in the literature (2). We show that the degradation/rearrangement of fosinopril is caused by several metal ions, in particular magnesium. A mechanism invoking metal chelation is proposed for the degradation of fosinopril sodium by this process. The kinetics of the metal ion-mediated degradation were studied by reacting fosinopril sodium with magnesium acetate tetrahydrate in methanol. The kinetic study established that the metal ion-mediated degradation was a second-order reaction between fosinopril and metal ion. The study helped to explain the degradation of fosinopril sodium in a prototype tablet formulation containing magnesium stearate as the lubricant.

MATERIALS AND METHODS

Materials

Fosinopril sodium was synthesized at Bristol-Myers Squibb Co. The following metal acetates were obtained from Aldrich Chemical Co.: magnesium acetate tetrahydrate, zinc acetate dihydrate, cobalt(II) acetate tetrahydrate, nickel(II) acetate tetrahydrate, barium acetate, and calcium acetate hydrate. The following metal acetate salts were obtained from Fischer Chemical Co.: potassium acetate, sodium acetate trihydrate, copper(II) acetate, and lithium acetate. Magnesium stearate and iron(II) chloride X · H_2O was obtained from Mallinkrodt, Inc. Iron(III) chloride hexahydrate was obtained from J. T. Baker Chemical Co. All the salts were used as received from the manufacturer. All solvents were of HPLC grade and reagents of analytical purity. The names fosinopril and fosinopril sodium are used synonymously and interchangeably.

Degradation of Fosinopril Sodium by Metal Acetates in Methanol

Fosinopril sodium was dissolved in methanol at a concentration of 0.0017 *M* and reacted with each of the metal

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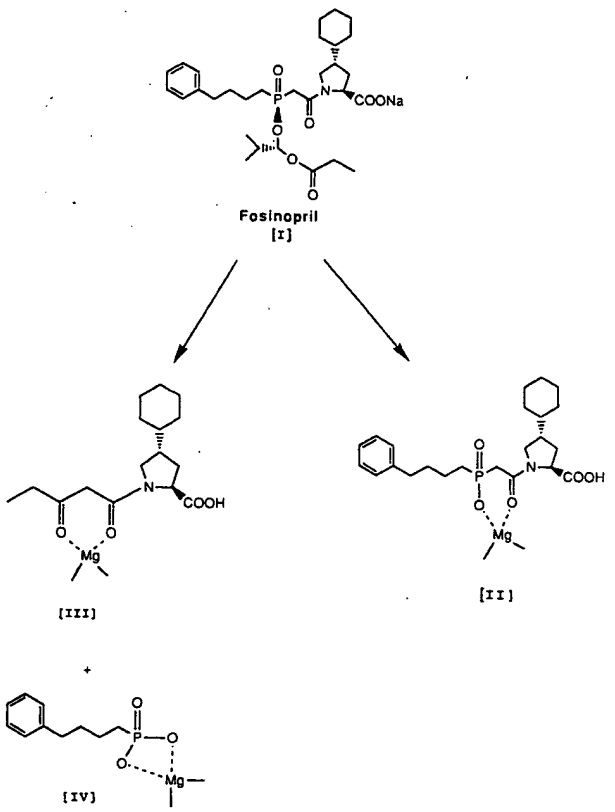


Scheme I. Pathways for degradation of fosinopril sodium. The degradation products II, III, and IV are shown in the form of magnesium ion chelates.

acetates at the same concentration. Acetate salts of calcium, barium, and manganic and magnesium stearate did not dissolve completely in methanol and were used as suspensions. The Fe(II) and Fe(III) salts were reacted as chlorides with potassium acetate added as a base. The reaction was allowed to proceed at 24°C (±1°C) for a specified time and then the contents of the flasks were withdrawn and analyzed by HPLC. Blank controls were solutions of fosinopril sodium in methanol at the same concentration. The data from these experiments were used to rank order the reactivity of each metal ion by calculating the pseudo-first-order rate constants for the degradation of fosinopril sodium.

Kinetics of Degradation of Fosinopril Sodium in the Presence of Magnesium Acetate in Methanol

For kinetic experiments equimolar stock solutions of fosinopril sodium (MW 585.6) and magnesium acetate tetrahydrate (MW 214.5) were prepared by dissolving separately 100 and 36.5 mg, respectively, in 100 mL of methanol. The two reactant solutions were mixed in a predetermined ratio in Teflon stoppered flasks and appropriately diluted with methanol. A series of flasks were then placed in a constant-temperature bath at 24°C (±1°C) and samples were periodically withdrawn and analyzed by HPLC.

Positive control experiments were performed by reacting fosinopril sodium with potassium acetate (anhydrous) or sodium acetate trihydrate dissolved in methanol in a similar manner. Solutions of fosinopril sodium in methanol without any other additive served as blank controls. The data from these experiments were fitted to a second-order kinetic model (Table II).

Effect of Additives on Magnesium Ion-Mediated Degradation of Fosinopril

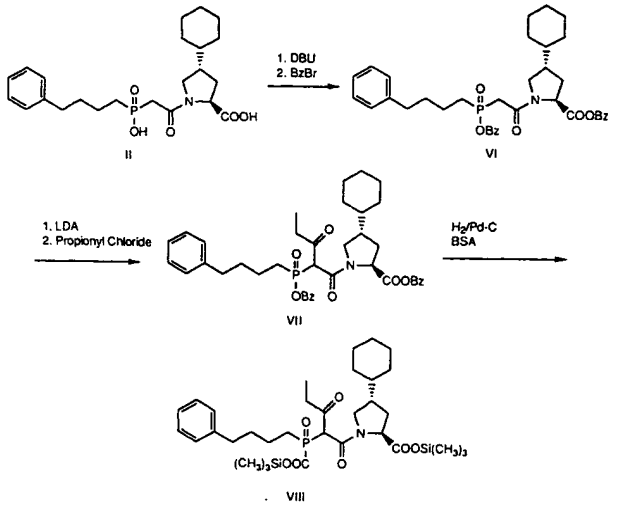
The effect of the II, III, and IV on the magnesium ion-mediated degradation of fosinopril was studied in methanol. Each additive was separately added to a methanolic solution of fosinopril sodium, followed by magnesium acetate in methanol. The molar ratio of the reactants was 1:1:1. The solutions were then placed in a constant-temperature bath at 24°C (±1°C). At periodic intervals, samples were withdrawn and analyzed by HPLC for fosinopril.

Synthesis of the Bis-Silylated Derivative of Intermediate (V)

The dibenzyl ester VI was prepared from the diacid II using DBU (1,8 diazabicyclo[5.4.0]undec-7-ene) and benzyl bromide (Scheme II). Compound VI was deprotonated with LDA (lithium diisopropylamide) and acylated with propionyl chloride to give the phosphinyldicarbonyl compound VII. Attempted hydrogenolysis of VII afforded an intractable mixture of compounds, however, in the presence of BSA [*N,O*-bis(trimethylsilyl)acetamide], provided the bis-silylated derivative VIII of the key intermediate V. The compound VIII was characterized by mass spectroscopy: ions at m/z = 564 [M + 1 − one $Si(CH_3)3]^+$, 492 [M + 1 − two $Si(CH_3)3]^+$, 562 [M − 1 − one $Si(CH_3)3]^-$, and 490 (M − 1 − two $Si(CH_3)3]^-$. ^{31}P-NMR of VIII gave a signal at δ 35.9.

Synthesis of III

β-Ketoamide, III, was obtained by condensation of 4-cyclohexylproline with 3-oxopentanoic acid in the presence of anhydrous 1-hydroxybenzotriazole (HOBT) and *N,N*-dicyclohexylcarbodiimide (DCC) in methylene chloride. The product was isolated as the dicyclohexylammo-



Scheme II. Synthesis of silylated derivative of the intermediate (V).

nium salt, purified by crystallization from methyl isobutyl ketone, and characterized by spectroscopy; m.p. 181°C, *Anal.* Calc. for $C_{28}H_{48}N_2O_4$: C, 70.55; H, 10.16; N, 5.88. Found: C, 70.28; H, 10.11; N, 5.78. MS $(M + H)^+ = 477$. IR (KBr): 2920 (s), 1630 (s), 1655 (m) cm^{-1}. ^{13}C-NMR ($CDCl_3$): carbonyl at δ 205.3, 175.8, and 166.0.

^{31}P-NMR Spectroscopy

^{31}P-NMR spectra were obtained at 360 MHz on a JOEL FX-90Q spectrometer using a 5-mm omni probe. Spectral acquisition parameters were 16K points, 6000 Hz (175 ppm), 2-sec repetition rate, and 30° pulse angle. A phosphoric acid external standard served as reference. Eighty pulse spectra were recorded.

^1H-NMR Spectroscopy

Proton spectra were obtained at 400 MHz on a JOEL GX-400 NMR spectrometer with a 5-mm proton probe. Spectral acquisitions parameter were 32K time domain data points, 600-Hz spectral width, 4-msec pulse (90° pulse, 10.5 m · sec), 4.7-sec repetition rate, and 0.2-Hz line broadening factor. A typical spectrum required 40 to 120 pulses to achieve the desired signal-to-noise ratio. All spectra were run at 30°C and referenced to internal TMS standard.

Mn(II) EPR Spectroscopy

EPR spectra were recorded on a Varian Model E-12 spectrometer operating nominally at 9.5 GHz and at 100-kHz field modulation frequency. Concentration standards of Mn(II) at 5 and 10 m*M* were made from reagent-grade Mn(II) acetate tetrahydrate in methanol. The EPR spectra were compared with spectra recorded at the same concentration after the addition of equimolar fosinopril sodium. Spectra were recorded at ca. 80 K using a gaseous nitrogen cryostat.

Fast Atom Bombardment (FAB) Mass Spectroscopy

Mass spectra were obtained on a double-focusing magnetic sector mass spectrometer (Model ZAB-1F, VG Analytical) by fast atom bombardment using a 4- to 8-kV xenon source. The sample was dissolved in methanol and taken up in the FAB matrix consisting of a mixture of dithiothreitol, dithioerythreitol, DMSO, and glycerol.

LC-MS Analysis

LC/MS analysis of the reaction mixture of fosinopril sodium and magnesium acetate at a 1:1 molar ratio in methanol at 24°C was carried out using a SCIEX API-III liquid chromatograph/mass spectrometer. A Waters LC/MS gradient controller system with simultaneous UV detection was employed for the chromatographic run. The eluant was split 20:1, with approximately 60 μL/min directed to the mass spectrometer. The ionization of the eluent was by nebulizer-assisted electrospray. Ions were produced with little internal energy, resulting in intact $(M + H)^+$ and $(M - H)^-$ ions.

HPLC Analysis

The HPLC system consisted of pump (Model 400, Applied Biosystems, Foster City, CA), an automatic sample processor (Model WISP712, Waters Associates, Milford, MA), and a UV detector (Model 783, Applied Biosystems). Data acquisition and analysis were performed using an HP-1000 computer system (Model A900 with RTE-A and CALS PeakPro Chromatography System, Beckman Instruments, Inc., Allendale, NJ). The chromatographic separations were performed on a phenyl column (Type C-402, 4.6 mm × 30 cm, 10-μm packing, Column Resolution Inc., San Jose CA) using a mixture of methanol and aqueous 0.2% phosphoric acid (72:28). Mobile phase was pumped at a flow rate of 1.5 mL/min. The wavelength of detection was 220 nm. In the experiments using LC-MS analysis, the mobile phase was methanol and aqueous 0.2% trifluoroacetic acid (72:28) pumped at a flow rate of 1.2 mL/min.

Thin-Layer Chromatography (TLC)

TLC of the reaction mixture of fosinopril sodium with magnesium acetate in methanol was carried out using a stationary phase plate of silica gel-G (Merck, G-60) and a mobile phase of chloroform:methanol:water (60:45:10). The temperature of the chamber was 5°C (±1°C). A reaction mixture containing an equimolar ratio of fosinopril sodium and magnesium acetate was initially monitored by HPLC analysis. After 30 min at room temperature the degradant appeared at an R_f of 0.65 when visualized by iodine vapors. The reaction mixture was then streaked across plates and a front equivalent to 16 cm was developed. A zone corresponding to R_f 0.6–0.7 was scrapped off the plates without visualization and extracted in methanol. The methanol extract was analyzed by fast atom bombardment mass sectroscopy. A similar procedure was followed for the isolation and characterization of degradation product from the tablets that were subjected to accelerated stability testing.

Molecular Modeling

Computer-generated space filling models of the fosinopril metal ion complex were elicited by using ALEX software (written by Jack Z. Gougoutas of Bristol-Myers Squibb) on an Iris 40/25 Super Turbo (Silicon Graphics) work station.

RESULTS

Degradation of Fosinopril Sodium in Methanol by Metal Ions

Figure 1 shows chromatograms of an equimolar mixture of fosinopril sodium and magnesium acetate in methanol. It shows that after a reaction time of 2 hr at 24°C, degradation of fosinopril formed an intermediate degradation product (Fig. 1B). Further heating at 37°C (±1°C) for 12 hr resulted in the formation of II, III, and IV (Fig. 1D). Identity of II, III, and IV was established by comparison to authentic samples. Also shown in Fig. 1 (A and C) are the chromatograms of an equimolar reaction mixture of fosinopril and magnesium stearate after similar treatment in methanol. From the products formed, it was confirmed that both magnesium stearate and magnesium acetate reacted with fosinopril in a similar manner. Other metal ion acetates exhibited similar reactivity towards fosinopril. The relative reactivities of different metal ions are shown in Table I. The reactivities were com-

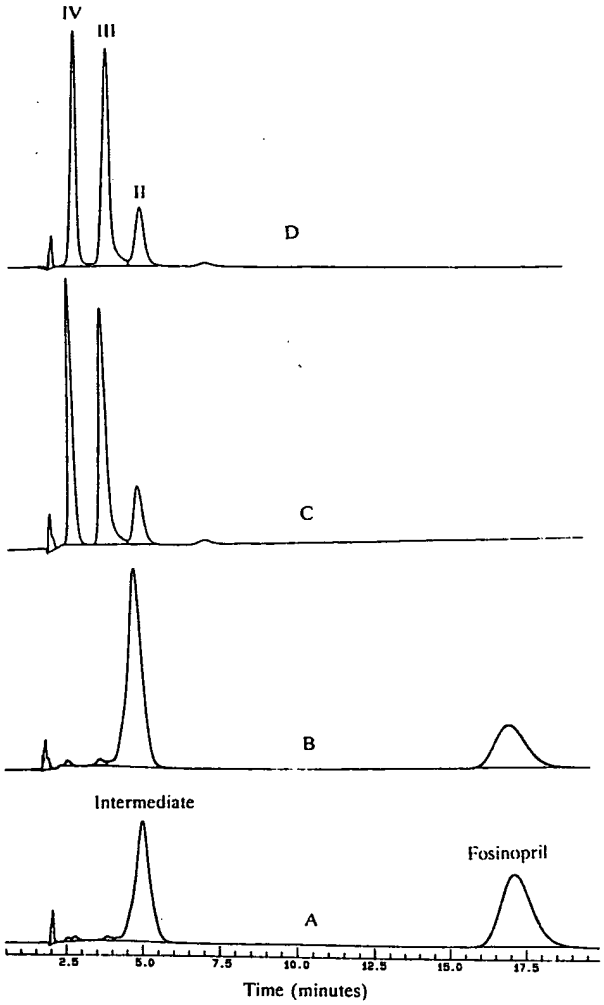


Fig. 1. Chromatograms of reaction mixtures containing 1:1 molar mixtures of fosinopril sodium in methanol, with magnesium stearate (A) at 24°C for 2 hr and (C) at 37°C for 12 hr and with magnesium acetate (B) at 24°C for 2 hr and (D) at 37°C for 12 hr.

pared by measuring the loss of fosinopril caused by each metal ion over a specified time period under identical conditions and then expressing the result as the pseudo-first-order rate constant for each metal ion. It was not clear whether the observed differences were due to inherent activating abilities of the metal ions or to their association constants with fosinopril. On a relative basis, Co(II) and Mn(II) were more reactive than Mg(II), whereas Zn(II), Ni(II), Mn(III), Cu(II), Ca(II), and Ba(II) were less reactive. Reactivity of Fe(III) and Fe(II) ions was determined by reacting their chloride salts since the acetates were not readily available. In these cases, potassium acetate was added as the base. Under the same conditions Fe(II) chloride did not cause degradation of fosinopril, whereas Fe(III) chloride was reactive. Sodium, potassium, and lithium acetates did not cause degradation of fosinopril.

Influence of Magnesium Ions on the Rate of Degradation of Fosinopril Sodium

Figure 2 shows the effect of varying the magnesium acetate concentration on the degradation of fosinopril sodium from methanolic solution at 24°C. It was observed that a greater loss of fosinopril occurred as the concentration of magnesium acetate was increased and that, after a fixed amount of degradation, the reaction appeared to level off. Likewise, when the reaction was carried out in the presence of a fixed concentration of magnesium acetate and varying fosinopril concentrations, a fixed amount of fosinopril proportional to the metal ion concentration was lost. Thus when the molar ratio of fosinopril:magnesium ion was changed from 1:1 to 1:0.5, the rate of degradation did not change but the reaction stopped after half the drug was consumed. Control experiments with sodium acetate or potassium acetate showed a negligible loss of fosinopril. These results indicated that the degradation of fosinopril by magnesium ions was a bimolecular process. The second-order rate constants at 24°C in methanol at different molar ratios of fosinopril to magnesium ion are shown in Table II. The rate constant did not deviate significantly upon varying the molar ratio of the reactants.

Table I. Relative Reactivities of Metal Ions in the Degradation of Fosinopril in Methanol at 24°C

Metal ion	% fosinopril remaining at 2.75 hr	Rate (hr^{-1})
Co(II)	13	0.74
Mn(II)	16	0.69
Mg(II)	19	0.60
Zn(II)	30	0.44
Ni(II)	31	0.43
Mn(III)	40	0.33
Cu(II)	45	0.29
Fe(III)	69	0.13
Ca(II)	69	0.13
Ba(II)	92	0.03

Differentiation of Hydrolytic and Magnesium Ion Pathways of Degradation of Fosinopril Sodium in Methanol

In order to establish the mass balance between the amount of fosinopril lost and the amounts of degradation products formed, a 1:2 molar mixture of fosinopril sodium and magnesium acetate in methanol was reacted at 50°C (±1°C) for 24 hr and II, III, and IV formed in the mixture

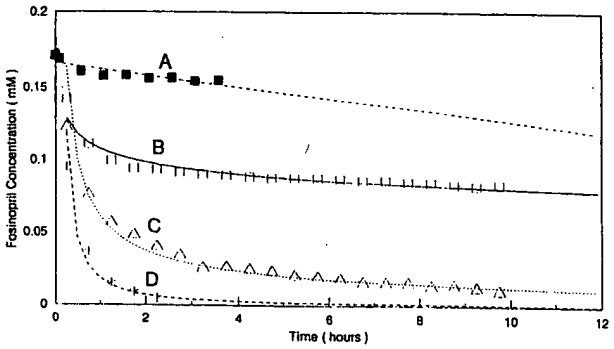


Fig. 2. Degradation of fosinopril sodium in methanol at 24°C, with a varying magnesium acetate-to-fosinopril mole ratio: (A) 0.1; (B) 0.5; (C) 1.0; (D) 1.5.

Table II. Second-Order Rate Constants for the Degradation of Fosinopril at Varying Ratios of Magnesium Acetate in Methanol at 24°C

Initial molar ratio of Fos:Mg^{2+}	Rate ($mmol^{-1}r^{-1}$) (SD)[a]
1:0.1	7.1 (*1.7*)
1.0.5	7.3 (*2.3*)
1:1	9.7 (*1.1*)
1:1.5	9.4 (*0.5*)
1:2	7.6 (*1.1*)

[a] Number of observations = 3.

were quantitatively determined (Table III). Control experiments were performed in which magnesium acetate tetrahydrate was replaced by either sodium acetate or potassium acetate to which known quantities of water were added. The distinct role of magnesium ion and water on the direction of the reaction pathway was established from the product profile. The formation of III and IV was attributed to magnesium ion pathway (88%), while II was formed by the hydrolysis (12%) of fosinopril caused by the water from the tetrahydrate salt form used.

Effect of Degradation Products on Reaction Kinetics

The second-order rate constants for the degradation of fosinopril by magnesium acetate at 24°C in methanol in the presence of the degradation products II, III, and IV, added separately, are shown in Table IV. The rate constants were calculated by monitoring the loss of fosinopril as a function of time. The magnesium ion-mediated degradation of fosinopril was significantly retarded in the presence of II, III, or IV.

Mass Spectral Analysis of the Reactive Intermediate

A reaction mixture containing mixture of fosinopril sodium and magnesium acetate at 24°C was subjected to tandem HPLC-MS analysis. The peak corresponding to "intermediate" (Fig. 1) gave ions at m/z = 492 $[M + H]^+$ and 514 $[M + Na]^+$. High-resolution mass spectral analysis (FAB) on a TLC isolate of the intermediate gave a negative ion at m/z = 512.2181 that was assigned to $[M - 2H + Na]^-$. The calculated value suggested an empirical formula of $[C_{26}H_{36}NO_6PNa]$ for the negative ion (theory = 512.2178). This corresponds to a formula of $C_{26}H_{38}NO_6P$ for the neutral molecule, consistent with the proposed structure of the intermediate V (Scheme III).

^{1}H-NMR Spectroscopy of the Reaction Mixture of Fosinopril Sodium and Magnesium Acetate

The methylene protons on C-7 of fosinopril in CD_3OD appear as a multiple at δ 2.85–3.25 (Fig. 3). After the addition of magnesium acetate to the solution, this signal was rapidly depleted. The double quartet at δ 6.25–6.35 due to the single side-chain methine proton on C-12 of fosinopril was also progressively diminished in the presence of magnesium acetate, as a result of the loss of side chain. On reaction with magnesium acetate in CD_3OD, fosinopril liberated isobutaraldehyde as its hemiacetal (doublet at δ = 4.16, $J \leq 8$ Hz). A small signal for free aldehyde proton was also detected at δ 9.5. With time, more isobutaraldehyde formed as detected by NMR. Formation of isobutyraldehyde was confirmed by spiking. The spectral pattern became complex after further standing, indicative of the formation of several compounds in the solution.

EPR Spectroscopy of the Reaction Mixture of Fosinopril Sodium and Manganese(II) Acetate in Methanol

The EPR spectrum of a reaction mixture containing equimolar Mn(II) acetate and fosinopril sodium in methanol is shown in Fig. 4B. It is shown that the peak height of the signal due to Mn(II) is three times greater than the intensity of the Mn(II) acetate alone in methanol (Fig. 4A) at the same concentration. The line shape is narrower, and "forbidden" hyperfine (ΔM_S = 1 and ΔM_I = 1) transitions exist for the initial complex which are not resolved in the broad spectrum of Mn(II) acetate control.

^{31}P-NMR Spectroscopy of the Reaction Mixture of Fosinopril Sodium and Magnesium Acetate

The ^{31}P resonance of fosinopril sodium in CD_3OD occurs as a doublet centered at δ 58.55. Immediately after adding magnesium acetate to the solution, the phosphorous signal appeared as a singlet at δ 58.6 (Fig. 5). After the reaction mixture remained at room temperature, another signal appeared at δ 36.1, which was assigned to the "intermediate" as indicated by the HPLC analysis of the sample.

DISCUSSION

In extended stability studies of the bulk drug substance, fosinopril sodium does not undergo the postulated rearrangement and degradation reactions. If exposed to high humidity, the ester prodrug undergoes hydrolysis to form the active moiety II. In the formulations containing magnesium stea-

Table III. Contributions of Hydrolysis and Magnesium Ion Pathways in the Degradation of Fosinopril in Methanol at 50°C

Addition	Fosinopril lost (%)[a]	Degradation products formed (%)		
		II	III	IV
None	1	Trace	Trace	Trace
Sodium acetate trihydrate	6	6	Trace	Trace
Potassium acetate anhydrous	1	1	Trace	Trace
Sodium acetate + $4H_2O$	14	11	2	Trace
Magnesium acetate tetrahydrate	100	12	88	88

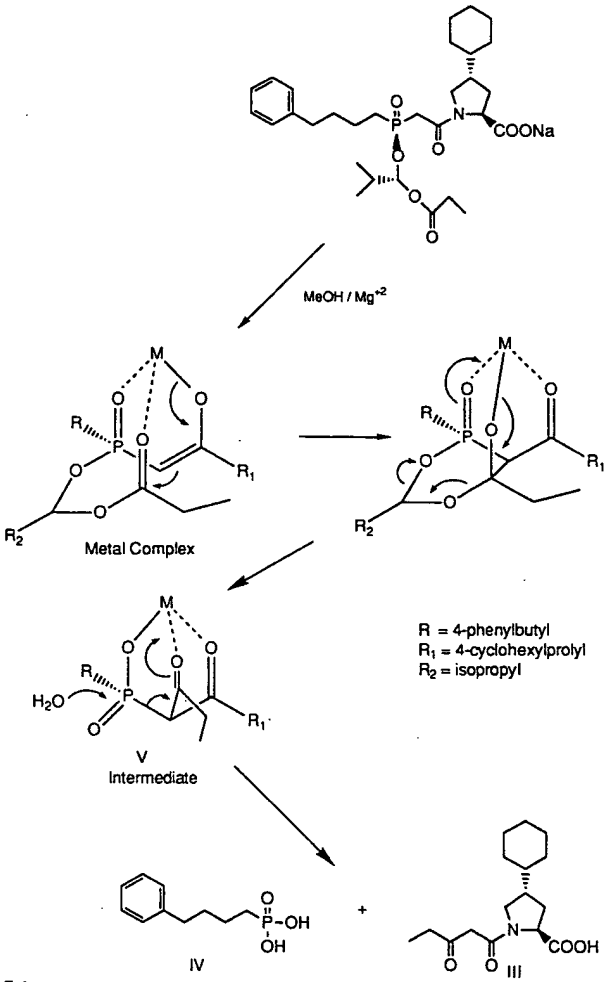
[a] Initial concentrations: fosinopril, 3.4×10^{-4} mmol/mL; metal acetates, 6.8×10^{-4} mmol/mL].

Table IV. Effect of II, III, and IV on Magnesium Ion-Mediated Degradation of Fosinopril in Methanol at 24°C[a]

Addition	Rate ($mmol^{-1}$ hr^{-1})
None	9.7
III	2.2
IV	1.5
II	0.006

[a] Molar ratio:fosinopril: magnesium acetate:degradant, 1:1:1.

rate, fosinopril degrades to form not only II, but also small amounts of III and IV. We have studied the degradation of fosinopril in a model system wherein the fosinopril was reacted with a soluble salt form of a metal in methanol. The solid-state behavior of fosinopril in tablet formulation was simulated in solution by substituting freely soluble magnesium acetate for magnesium stearate. We have demonstrated that the reaction was not unique to magnesium ions but occurred with other metal ions as well. There are several advantages to studying the incompatibility between fosinopril and metal ions in a solution system. The reaction could be monitored more accurately and with a precision many fold greater than the solid state. The solution model offers the flexibility of varying the reactant ratios and temperature and allows for the introduction of extraneous additives with minimal changes in the overall setup. Most importantly the reaction could be selectively directed toward the magnesium ion pathway, while minimizing the hydrolytic degradation of the ester prodrug moiety. The kinetic and mechanistic details of the metal ion-mediated reaction of fosinopril are given below.



Scheme III. Proposed mechanism for the degradation of fosinopril sodium by metal ion.

Directional Control of the Reaction in Methanol as Solvent

Scheme I shows the two pathways postulated for degradation of fosinopril sodium. The ester prodrug is shown to degrade by hydrolysis to form the active agent II and by the metal ion-mediated pathway to III and IV. Dependence of the reaction pathway on the presence of metal ions or water was demonstrated by studying the process in methanol, in which known amounts of water or metal salts were introduced and then heated at 50°C for 24 hr (Table III). Under the experimental conditions 100% of fosinopril degraded in the presence of 2 equiv of magnesium acetate tetrahydrate to form 12% of II, 88% of III, and 88% of IV, whereas in the presence of potassium acetate only 1% of the drug was degraded, forming II. These observations suggested that the formation of III and IV was caused by magnesium ions. When sodium acetate trihydrate was added, it caused a 6% loss of fosinopril, resulting in the formation of II as the major product. When the reaction with anhydrous sodium acetate was carried out in the presence of 4 equiv of water the loss of fosinopril was increased to 14%, with II again being the major product. Thus it was concluded that in the presence of magnesium acetate tetrahydrate, II was formed due to hydrolysis, whereas the formation of III and IV was metal ion mediated. The small amount of III and IV formed in these control experiments in the absence of added magnesium ions was attributed to the presence of trace metal impurities contributed by the system. Formation of equal amounts of III and IV is significant and consistent with the proposal of the reaction proceeding through an "intermediate" because III and IV can be viewed as the molecular fragments of the intermediate (Scheme III).

Order of the Reaction and Self-Limiting Kinetics

Reactivity of magnesium ions towards fosinopril sodium was increased in methanol as a solvent. In control experiments with an equimolar mixture with magnesium acetate 100% of the initial fosinopril was lost within 2 hr at 50°C in methanol, whereas under the same experimental conditions in water less than 1% loss was observed. Thus, using methanol as the solvent allowed the reaction to progress through several half-lives in a short period of time, facilitating accurate assessment of the kinetics of the process. The kinetic data were fitted to a second-order model:

$$\ln[B_t/A_t] = \ln[B_0/A_0] + (B_0 - A_0)\,kt$$

where A_0 and B_0 are the initial concentrations of fosinopril sodium and magnesium acetate and A_t and B_t are the residual concentrations at time t. Though the rate of the reaction is

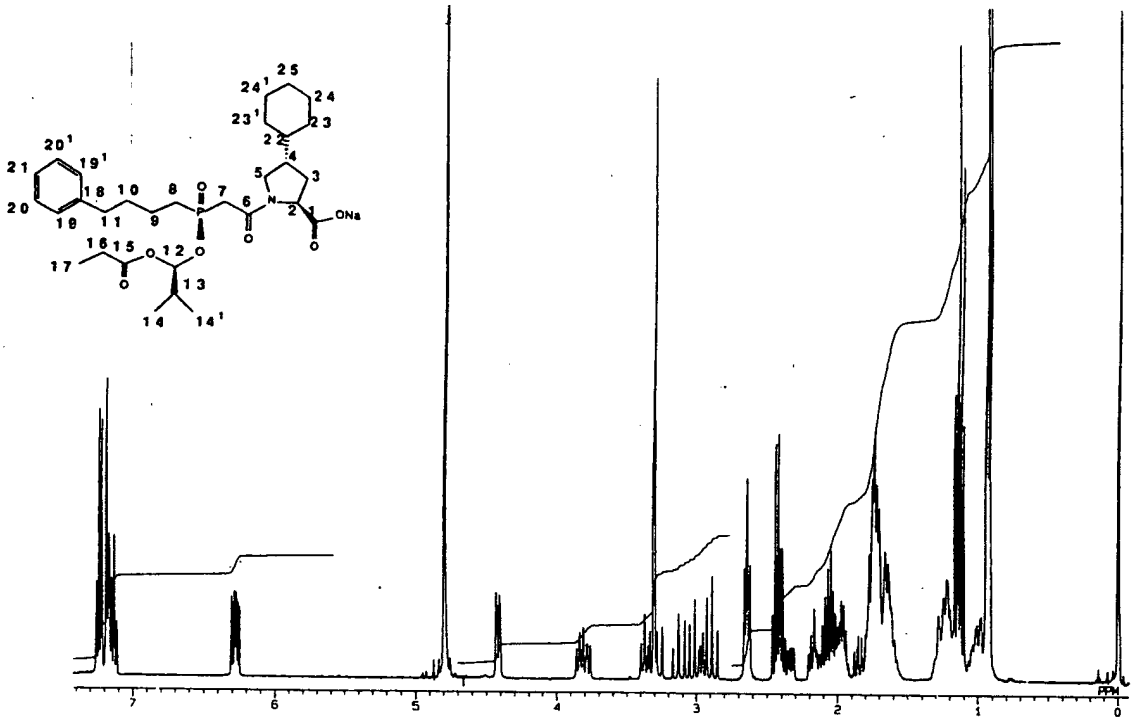


Fig. 3. ^{1}H-NMR spectrum of fosinopril sodium in CD_3OD.

dependent on the concentration of both fosinopril and magnesium acetate, only the fosinopril concentration was actually measured as a function of time. The actual concentration of magnesium ions remained unchanged as a function of time, though their reactivity toward fosinopril was decreased as shown by the leveling of the reaction with time when less than 1 equiv of magnesium acetate was used. Since the initial concentration of both fosinopril sodium and magnesium acetate was known, the effective concentration of magnesium ions could be calculated (3). The rate constants calculated from these data are shown in Table II. It can be seen that the

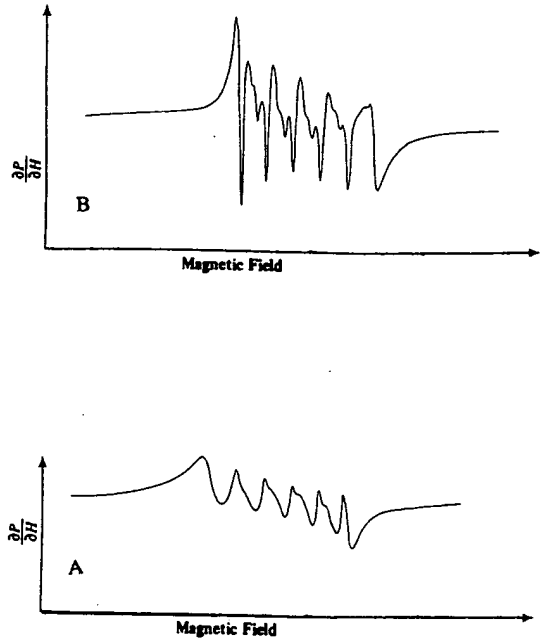


Fig. 4. EPR spectrum: (A) Mn(II) acetate (10 m*M*) in methanol and (B) Mn(II) acetate (10 m*M*) in the presence of equimolar fosinopril sodium in methanol.

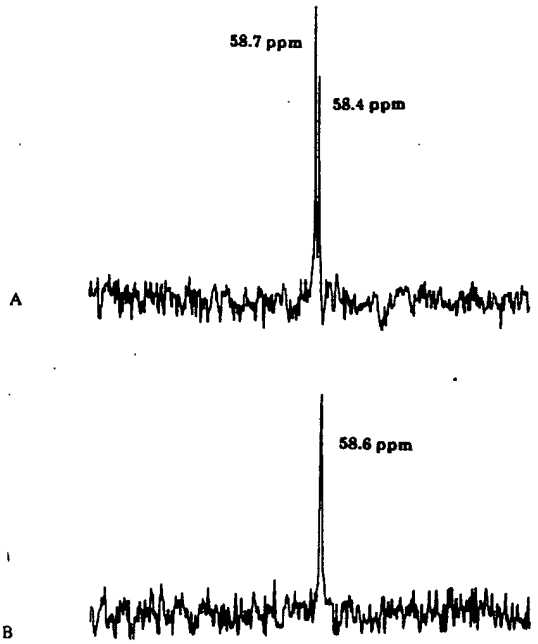


Fig. 5. ^{31}P-NMR spectrum: (A) fosinopril sodium in CD_3OD and (B) after the addition of equimolar molar magnesium acetate in CD_3OD.

value of the rate constant remained relatively invariant even when the ratio of the two reactants was varied over a large range. The second-order kinetics demonstrated the stoichiometric rather than catalytic involvement of magnesium ions in the reaction. This was, at first thought, surprising because the concentration of magnesium ions in the solution does not change with time. However, II, III, and IV are all capable of complexing magnesium ions, hence it is likely that their formation would retard the reaction rate due to removal of the metal ions from further reaction. According to this model, the magnesium ion-mediated degradation of fosinopril is a self-limiting process (Scheme I) and the degradation will "level off" with time in the presence of an excess of fosinopril. This is an unusual instance of incompatibility of a pharmaceutical entity in which there is a first-order dependence of the reaction on the availability of the metal ion. Most reported incompatibilities of drug substances with metal ions in the literature describe the catalytic involvement of metal ions. The reported example of reactions that show leveling trends are usually attributed to reduced availability of reactants such as moisture or oxygen (4–6). However, these examples differ significantly from the present case. In the magnesium ion-mediated degradation of fosinopril, the rate of the reaction is initially promoted by the metal ion but is progressively slowed down due to the effective removal of the reactive metal ions by the products of the reaction. Such involvement of metal ions in the hydrolysis of substrates of biological interest is well documented (7). Self-limiting reaction kinetics are described for Fe(III)-promoted hydrolysis of diester prodrugs of iron chelating agents *N,N'*-bis(2-hydroxybenzyl) and *N,N'*-bis(2-hydroxyphenyl)ethylenediamine diacetic acid by Pitt *et al.* (8). The authors report that overall rate of hydrolysis was second order with respect to both the ligand and the metal ion concentration. We have shown that the degradation of fosinopril is similarly affected in the presence of metal ions.

Mechanism

The mechanistic steps of the metal ion mediated reaction of fosinopril leading to III and IV are presented in Scheme III. A central feature of this rearrangement is the ability of the phosphodicarbonyl system of fosinopril to form an activated complex with the metal ion. A computer-generated complex between fosinopril and magnesium ion is shown in Figs. 6A and B). The structures were generated by starting with the conformation (translational atomic coordinates) from the solved single crystal structure of fosinopril sodium (9). The geometry was then adjusted by rotating bonds and changing the angles to correspond to the hypothesized structure of the magnesium ion complex. The space filling model displays the appropriate van der Waal radii of the constituent atoms. The stereo line drawing shows the enol form of the proposed complex with half-van der Waal radii representation of the sp^2 orbitals at the reactive centers and for the magnesium ion. All the manipulations resulted in allowable conformations with respect to orbital overlap and bond angles.

Involvement of the phosphinyl–dicarbonyl system in the metal complex was suggested from the results of the ^{31}P NMR and Mn(II)EPR. The ^{31}P NMR spectrum of fosinopril sodium in CD_3OD gives two resonance signals at δ 58.7 and 58.4 (Fig. 4). This is due to two distinct conformations of the molecule around the amide bond. On the addition of magnesium ions, the phosphorous resonance occurs as a singlet centered at δ 58.6, implying that rotation around the amide bond freezes and a single conformation results due to complexation of the metal ion.

As shown in Table I, Mn(II) ions also caused the degradation of fosinopril. Therefore it was thought correct to draw inference on the fosinopril–metal interaction from EPR spectroscopy. The complex formation was studied by reacting Mn(II) acetate with fosinopril in methanol. Mn(II) has a nuclear spin quantum number $I = 5/2$ and hence gives a characteristic six-line EPR signal due to ^{55}Mn hyperfine structure. On the addition of fosinopril sodium the peak height of the Mn(II) signal in frozen methanol is increased by a factor of three, while the average line width decreases between 20 and 50% depending on the nuclear state ($M_I = -5/2$ to $+5/2$). The line shape and intensity changes indicate that fosinopril binds to Mn(II) in methanol. We estimate a dissociation constant of $K_D = 0.1$ mM, assuming a 1:1 complex. The sharpening of line width upon binding indicates that the coordination environment of Mn(II) acetate in methanol becomes more homogeneous. This feature is expected only if fosinopril binds to create a more uniformly ordered coordination site.

The key steps in the degradation of fosinopril by metal ions are the formation of the complex, followed by deprotonation to give the magnesium enolate. The enhanced acidity of the protons on the C-7 was demonstrated by NMR, wherein a rapid exchange was observed with the solvent on the addition of magnesium acetate. The complexation of the metal serves to bring the C-7 and C-16 into the correct spatial orientation for orbital overlap to occur in the bond forming process (Fig. 6B). The attack of the enolate C-7 on the carbonyl C-16 gives a six-member ring transition state which collapses to give the proposed reactive intermediate V (Scheme III). The formation of V in the solution was inferred from the chromatographic and spectral analysis. But its isolation as a solid was not successful. The literature on aldol reactions is replete with examples of metal ion involvement in facilitating attack of enolate on the carbonyl group (10,11). Solladie *et al.* report an stereoselective addition of an α-sulfinic ester to an aldehyde (12) that bears a similarity to the proposed reaction of fosinopril with metal ion. The strategy that Nicolaou *et al.* (13) have employed, utilizing the keto phosphonate–aldehyde system in the ring closure reaction in the synthesis of macrolides tylosin and ampholide, is cited in support of the reaction mechanism proposed for the degradation of fosinopril by metal ions. This transformation of fosinopril under the mediacy of metal ion bears a topographic resemblance to the enolate Claisen rearrangement (14) and related Carroll rearrangement (15).

The driving force for the rearrangement/degradation cascade is chelation of metal ion by fosinopril. The metal ion presumably facilitates the cleavage reaction through binding to the intermediate, providing a conformation which maximizes the orbital overlap thus lowering the overall reaction barrier. The hydrolysis of the intermediate V (Scheme III) by the attack of water at the phosphinyl group of the intermediate affords the products III and IV in equimolar quantities.

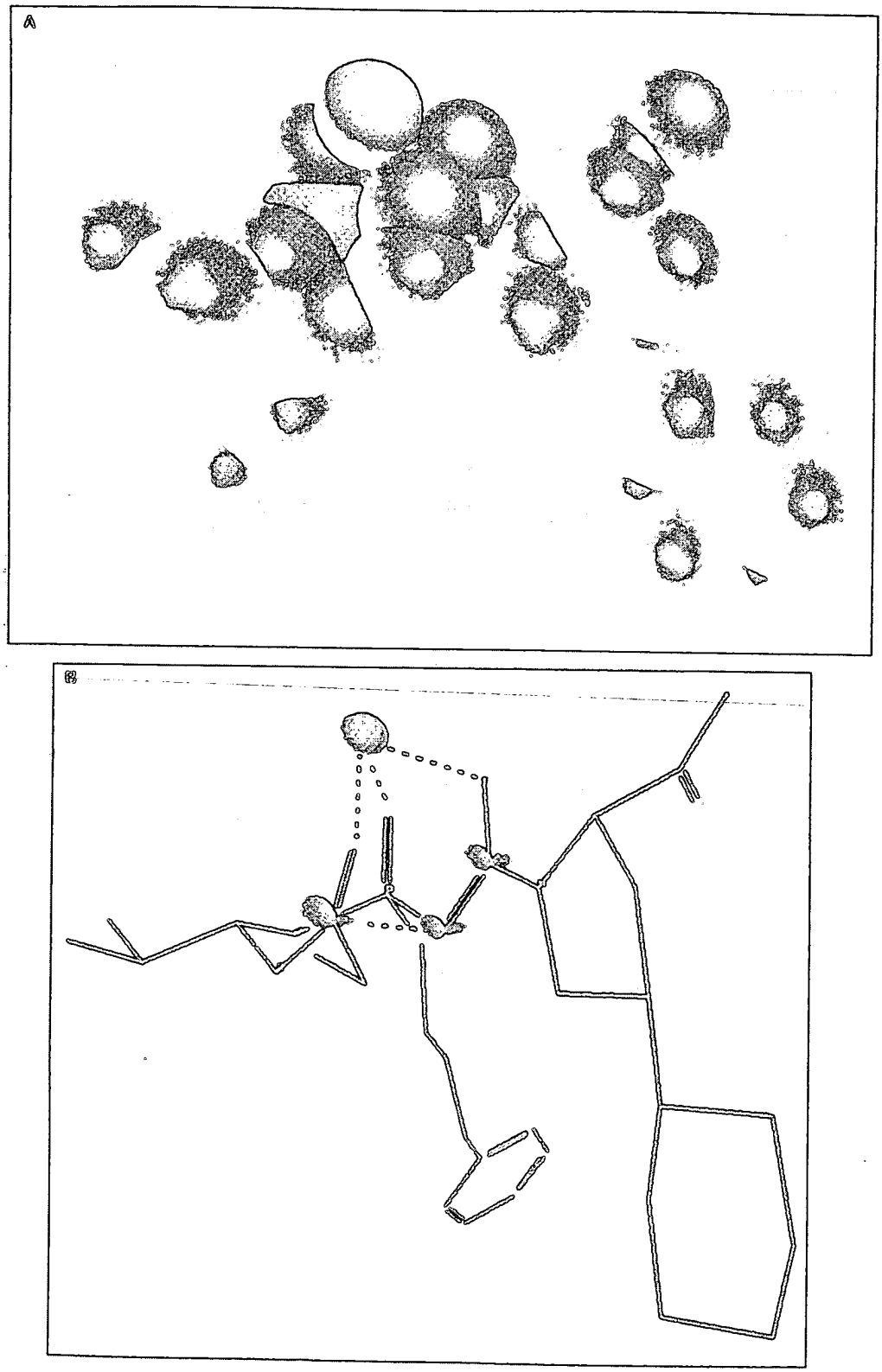


Fig. 6. Structural representation of interaction of fosinopril with magnesium ion. (A) Space filling model: magnesium ion (off white), phosphorus (yellow), oxygen (red), nitrogen (blue) and carbon (gray). (B) Stereo projections.

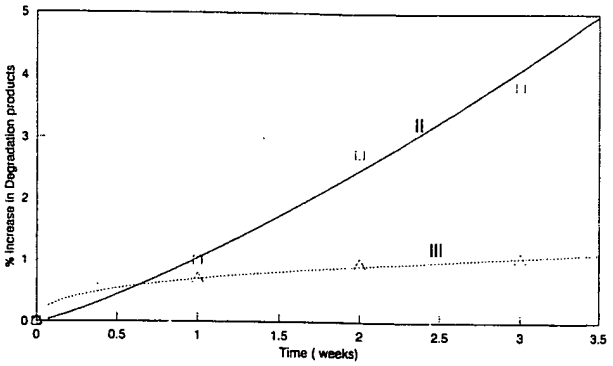


Fig. 7. Degradation of fosinopril sodium in a prototype tablet formulation containing magnesium stearate after storage at 50°C and 100% relative humidity, the showing formation of degradation products II and II as a function of time.

A notable feature of this reaction is the breaking of a carbon–phosphorous sigma bond under remarkably mild conditions. This process is analogous to metal-catalyzed dephosphonylation of 2-amino-3-phosphonopropionic acid by pyridoxal (16). The facile cleavage of the C–P bond is likely a result of the stabilization of the enolate of III by the metal ion. Thus the metal ion plays a central role in this transformation by bringing the respective reaction centers into the correct orientation and proximity. The net effect is the acylation of an active methylene group. The mediacy of the metal ion facilitating this transformation is reminiscent of enzyme mimetics (17,18). We intend to investigate the generality of this reaction with other molecules bearing similar structural features.

Significance to Tablet Formation

The results of this study helped rationalize the degradation of fosinopril in the tablet formulation lubricated with magnesium stearate. It clearly identified two distinct pathways of degradation, i.e., magnesium ion mediated and hydrolysis. In the tablet formulation the amount of the lubricant is low compared to drug and hence the magnesium ion-mediated degradation would occur only to a small extent as predicted by the second-order kinetic model. However, the formation of acidic degradation products would enhance the acid catalyzed degradation of the ester prodrug. In Fig. 7 stability data from an experimental fosinopril tablet formulation containing magnesium stearate as lubricant and stored at 50°C and 100% relative humidity are shown. The formation of magnesium ion-mediated product III levels off, whereas the formation of hydrolysis product II continues with time of storage. The data thus validate the predictions of the model.

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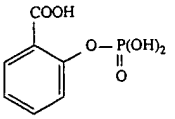
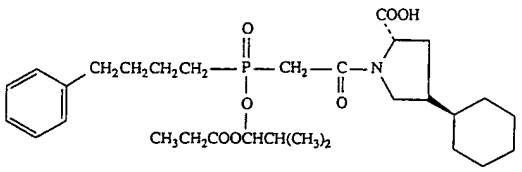


White solid, mp 168-170°. Sol in water, ethanol, acetone. Insol in non-polar organic solvents. Hydrolyzes in aq soln. LD_{50} in male, female mice, male, female rats at pH 1.0, aq soln (mg/kg): 94, 105, 153, 257 i.v.; 352, 253, 338, 360 i.p.; 1455, 1539, 1104, 1213 orally; at pH 3.5 (mg/kg): 117, 118, 207, 215 i.v.; 1592, 1483, 1085, 1128 i.p.; 1702, 2007, 1685, 2225 orally (Sanchez).

THERAP CAT: Analgesic.

4171. Fosinopril. *(2α,4β)-4-Cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline;* (4*S*)-4-cyclohexyl-1-[[[(*RS*)-1-hydroxy-2-methylpropoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline propionate (ester); fosenopril. $C_{30}H_{46}NO_7P$; mol wt 563.67. C 63.93%, H 8.23%, N 2.48%, O 19.87%, P 5.49%. Phosphinic acid containing angiotensin converting enzyme inhibitor. Prepn: E. W. Petrillo, Jr., U.S. pat. **4,337,201** (1982 to Squibb); of active diacid form: J. Krapcho *et al., J. Med. Chem.* **31**, 1148 (1988). Metabolism and pharmacokinetics: S. M. Singhvi *et al., Brit. J. Clin. Pharmacol.* **25**, 9 (1988). GC determn of diacid: M. Jemal *et al., J. Chromatog.* **345**, 299 (1985). Clinical trial in hypertension: P. A. Sullivan *et al., Am. J. Hypertension* **1**, 280S (1988). Brief description: E. W. Petrillo, Jr., *et al., Clin. Exp. Theory Prac.* **A9**, 235 (1987).

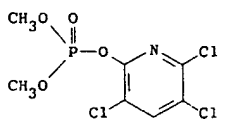


Sodium salt, $C_{30}H_{45}NNaO_7P$, *SQ 28555, Monopril.*

Diacid, $C_{23}H_{34}NO_5P$, *SQ 27519.* mp 149-153°. $[\alpha]_D$ −24° (c = 1 in methanol).

THERAP CAT: Antihypertensive.

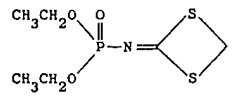
4172. Fospirate. *Dimethylphosphoric acid 3,5,6-trichloro-2-pyridinyl ester; O,O*-dimethyl-*O*-3,5,6-trichloro-2-pyridyl phosphate; Dowco 217; Torelle. $C_7H_7Cl_3NO_4P$; mol wt 306.46. C 27.43%, H 2.30%, Cl 34.70%, N 4.57%, O 20.88%, P 10.11%. Prepn by reaction of 3,5,6-trihalo-2-pyridinol with phosphoryl chloride: Rigterink, Kenaga, *J. Agr. Food Chem.* **14**, 304 (1966).



Crystals from petr ether, mp 86.5-88°.

THERAP CAT (VET): Anthelmintic.

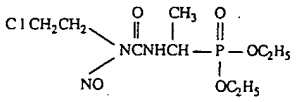
4173. Fosthietan. *1,3-Dithietan-2-ylidenephosphoramidic acid diethyl ester; phosphonodithioimidocarbonic acid cyclic methylene P,P-diethyl ester;* 2-(diethoxyphosphinylimino)-1,3-dithietane; AC 64475; CL 64475; Nem-A-Tak. $C_6H_{12}NO_3PS_2$; mol wt 241.26. C 29.87%, H 5.01%, N 5.81%, O 19.89%, P 12.84%, S 26.58%. Prepn: R. W. Addor, S. Afr. pat. **68 01,064** corresp to U.S. pat. **3,470,207** (1968, 1969 to Am. Cyanamid); *idem, J. Heterocycl. Chem.* **7**, 381 (1970). Activity: W. K. Whitney, J. L. Aston, *Proc. Brit. Insectic. Fungic. Conf.* **2**, 625 (1975).



Pale yellow oil, mercaptan-like odor. n_D^{25} 1.5348; d_{25} 1.3 Vapor press at 25°: 6.5 × 10^{-6} mm Hg. Soly in water at 25°: 50 g/kg. Sol in acetone, chloroform, methanol, toluene. LD_{50} orally in rats, mice: 5, 18 mg/kg, W. K. Whitney, J. L. Aston, *loc. cit.*

USE: Nematocide; insecticide.

4174. Fotemustine. *[1-[[[(2-Chloroethyl)nitrosoamino]carbonyl]amino]ethyl]phosphonic acid diethyl ester;* (±)-diethyl [1-[3-(2-chloroethyl)-3-nitrosoureido]ethyl]phosphonate; 1-[*N*-(2-chloroethyl)-*N*-nitrosoureido]ethylphosphonic acid diethyl ester; S-10036. $C_9H_{19}ClN_3O_5P$; mol wt 315.69. C 34.24%, H 6.07%, Cl 11.23%, N 13.31%, O 25.34%, P 9.81%. Amino acid-linked nitrosourea alkylating agent. Prepn: G. Lavielle, C. Cudennec, Fr. pat. 2,536,075; *eidem,* U.S. pat. **4,567,169** (1984, 1986 both to ADIR). Clinical evaluation in advanced cancers: D. Khayat *et al., Cancer Res.* **47**, 6782 (1987); in disseminated malignant melanoma: D. Khayat *et al., J. Nat. Cancer Inst.* **80**, 1401 (1988).



mp 85°.

THERAP CAT: Antineoplastic.

4175. Francium. Eka-cesium. Fr; at. no. 87. ^{223}Fr, *Actinium K,* is the most stable isotope ($T_{½}$ 21 min; β-emitter) formed by α-decay of actinium (^{227}Ac). Found in uranium minerals. First obtained in 1939 from an actinium prepn by Perey, *J. Phys. Radium* **10**, 439 (1939); *J. Chim. Phys.* **43**, 155, 262 (1946); Hyde, Ghiorso, *Phys. Rev.* **90**, 267 (1953); Hyde, *J. Am. Chem. Soc.* **74**, 4181 (1952). Isoln by paper chromatography: Perey, Adloff, *Compt. Rend.* **236**, 1161 (1953). Also obtainable by proton bombardment of thorium. Mass numbers of other known isotopes: 204-213; 217-222, 224. Most electropositive element. Chemical behavior similar to that of other alkali metals. *Reviews:* Hyde, *J. Chem. Ed.* **36**, 15-21 (1959); Whaley, "Sodium, Potassium, Rubidium, Cesium and Francium" in *Comprehensive Inorganic Chemistry* **vol. 1**, J. C. Bailar Jr., *et al.,* Eds. (Pergamon Press, Oxford, 1973) pp 369-529.

4176. Frangula. Buckthorn bark; alder buckthorn; black dogwood; berry alder; arrow wood; Persian berries. Dried bark of *Rhamnus frangula* L., *Rhamnaceae. Habit.* Europe, Russian Asia, Mediterranean coast of Africa. *Consists:* Frangulin, emodin, chrysophanic acid.

4177. Frangulin. Franguloside; avornin; Cascarin. In berries, bark, and rootbark of *Rhamnus* spp., especially in alder buckthorn *(Rhamnus frangula* L.), *Rhamnus carthartica* L., and *Rhamnus purshiana* DC. (*Cascara sagrada*). *Rhamnaceae.* Prepn from bark of alder buckthorn: Bridel, Charaux, *Bull. Soc. Chim. Biol.* **15**, 642 (1933); M. Kubisa, *Herba Pol.* **23**, 217 (1977), *C.A.* **88**, 166260b (1978). Consists of the two glucosides, frangulins A and B, which were originally thought to be isomeric. Structure and synthesis of frangulin A: Hörhammer, Wagner, *Z. Naturforsch.* **27B**, 955 (1972). Structure of frangulin B: Wagner, Demuth, *Tetrahedron Letters* **1972**, 5013.

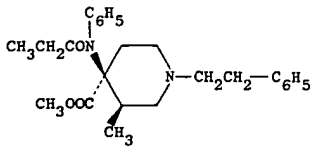
view: F. Rol in *Industrial Gums*, R. L. Whistler, Ed. (Academic Press, New York, 2nd ed., 1973) pp 323-337.

Yellow-green color, is odorless and tasteless, but acquires a leguminous taste when boiled in water.

USE: Stabilizer, thickener, and binder in foods and cosmetics. Coffee, chocolate, cocoa substitute. Sizing and finishing agent in textiles. As fiber bonding in paper manuf. Drilling mud additive.

THERAP CAT: Adsorbent-demulcent.

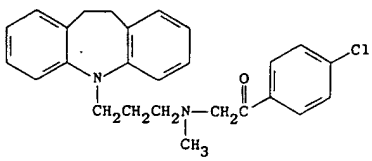
5437. Lofentanil. *3-Methyl-4-[(1-oxopropyl)phenylamino]-1-(2-phenylethyl)-4-piperidinecarboxylic acid methyl ester;* (−)-*cis*-3-methyl-1-phenethyl-4-(*N*-phenylpropionamido)isonipecotic acid methyl ester. $C_{25}H_{32}N_2O_3$; mol wt 408.55. C 73.50%, H 7.90%, N 6.85%, O 11.75%. Prepn: P. A. J. Janssen, G. H. P. Van Daele, Ger. pat. **2,610,228** corresp to U.S. pat. **3,998,834** (both 1976 to Janssen). Receptor affinity and pharmacological potency: K. D. Stahl *et al., Eur. J. Pharmacol.* **46,** 199 (1977). Structural study: C. De Ranter *et al., Arch. Int. Physiol. Biochim.* **87,** 1031 (1979). Antinociceptive effects in cats: A. S. Tung, T. L. Yaksh, *Pain* **12,** 343 (1982). Opiate receptor binding studies of ^{3}H-lofentanil: W. Gommeren, J. E. Leysen, *Arch. Int. Pharmacodyn. Ther.* **258,** 171 (1982); B. Ilien *et al., ibid.* 313; P. M. Laduron, P. Janssen, *Life Sci.* **31,** 457 (1982).



Oxalate, $C_{27}H_{34}N_2O_7$, ***R 34995.*** Solid, mp 177°.

THERAP CAT: Narcotic analgesic.

5438. Lofepramine. *1-(4-Chlorophenyl)-2-[[3-(10,11-dihydro-5H-dibenz[b,f]azepin-5-yl)propyl]methylamino]-ethanone; 4'-chloro-2-[[3-(10,11-dihydro-5H-dibenz[b,f]-azepin-5-yl)propyl]methylamino]acetophenone; N*-methyl-*N*-(4-chlorobenzoylmethyl)-3-(10,11-dihydro-5*H*-dibenzo-[*b,f*]azepin-5-yl)propylamine; lopramine. $C_{26}H_{27}ClN_2O$; mol wt 418.97. C 74.54%, H 6.50%, Cl 8.46%, N 6.68%, O 3.82%. Psychotropic drug related to imipramine, *q.v.* Prepn: E. Eriksoo *et al.*, Brit. pat. **1,177,525;** *eidem,* U.S. pat. **3,637,660** (1970, 1972 both to AB Leo). Chemistry and pharmacology: E. Eriksoo, O. Rohte, *Arzneimittel-Forsch.* **20,** 1561 (1970). Absorption and metabolism: J. R. Tulic *et al., Acta Pharmacol. Toxicol.* **32,** 304 (1973). Distribution and excretion: G. Plym Forshell, *Xenobiotica* **5,** 73 (1975). Pharmacokinetics: G. Plym Forshell *et al., Eur. J. Clin. Pharmacol.* **9,** 291 (1976). Clinical study: S. Wright, L. Herrmann, *Arzneimittel-Forsch.* **26,** 1167 (1976).

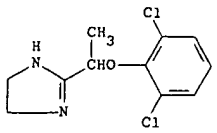


Crystals from methanol or acetone, mp 104-106°. Easily oxidized by air and other oxidizing agents to desipramine and *p*-chlorobenzoic acid.

Hydrochloride, $C_{26}H_{28}Cl_2N_2O$, ***Leo 640, Amplit, Gamanil, Gamonil, Timelit, Tymelyt.*** Crystals from butanone, mp 152-154°. Sol in methanol, ethanol, chloroform. Practically insol in water. LD_{50} in mice, rats (mg/kg): >2500, >1000 orally; 920, >1000 i.p.; >1000, >1000 s.c. (Eriksoo, Rohte).

THERAP CAT: Antidepressant.

5439. Lofexidine. *2-[1-(2,6-Dichlorophenoxy)ethyl]-4,5-dihydro-1H-imidazole; 2-[1-(2,6-dichlorophenoxy)ethyl]-2-imidazoline.* $C_{11}H_{12}Cl_2N_2O$; mol wt 259.13. C 50.99%, H 4.67%, Cl 27.36%, N 10.81%, O 6.17%. Vasoactive agent related structurally to clonidine, *q.v.* Prepn of the HCl salt: H. Baganz, H. J. May, S. Afr. pat. **68 00,850** corresp to U.S. pat. **3,966,757** (1968, 1976 both to Nordmark); of the free base: *eidem,* Ger. pat. **1,935,479** (1971 to Nordmark), *C.A.* **74,** 87979 (1971). Pharmacological studies: J. Velly, *J. Pharmacol.* **8,** 351 (1977); B. Jarrot *et al., Biochem. Pharmacol.* **28,** 141 (1979). NMR data and cardiovascular effects: P. B. M. Timmermans, P. A. Van Zwieten, *Eur. J. Med. Chem.* **15,** 323 (1980). Hypotensive and sedative properties: P. Birch *et al., Brit. J. Pharmacol.* **68,** 107 (1980). Effects in hypertension: N. D. Vlachakis *et al., Fed. Proc.* **39, 4844** (1980). Series of articles on pharmacology, toxicity studies, clinical studies: *Arzneimittel-Forsch.* **32,** 915-993 (1982). Toxicity: T. H. Tsai *et al., ibid.* 955.

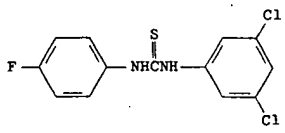


Cryst, mp 126-128°.

Hydrochloride, $C_{11}H_{13}Cl_3N_2O$, ***BA 168, MDL-14042A, Lofetensin, Loxacor.*** Cryst from ethanol/ether or 2-propanol, mp 221-223° (U.S. patent); also reported as mp 230-232° (Ger. patent). Very sol in water, ethanol. Slightly sol in 2-propanol. Practically insol in ether. LD_{50} in mice, rats, dogs (mg/kg): between 74-147 orally; between 8-18 i.v. (Tsai).

THERAP CAT: Antihypertensive.

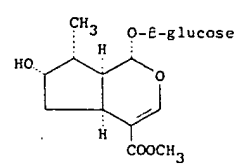
5440. Loflucarban. *N-(3,5-Dichlorophenyl)-N'-(4-fluorophenyl)thiourea; 3,5-dichloro-4'-fluorothiocarbanilide;* Fluonilid. $C_{13}H_9Cl_2FN_2S$; mol wt 315.21. C 49.53%, H 2.88%, Cl 22.50%, F 6.03%, N 8.89%, S 10.17%. Prepd from *p*-fluorophenyl isothiocyanate and 3,5-dichloroaniline or from 3,5-dichlorophenyl isothiocyanate and *p*-fluoroaniline: Belg. pat. **613,154** (1962 to Madan), *C.A.* **58,** 474f (1963).



Crystals from ethanol, mp 148°. Soluble in ethyl oleate, isopropyl myristate.

THERAP CAT: Antifungal.

5441. Loganin. *1-(β-D-Glucopyranosyloxy)-1,4a,5,6,7,7a-hexahydro-6-hydroxy-7-methylcyclopenta[c]pyran-4-carboxylic acid methyl ester;* 7-hydroxy-6-desoxyverbenalin. $C_{17}H_{26}O_{10}$; mol wt 390.40. C 52.30%, H 6.71%, O 40.99%. Key intermediate in the biosynthesis of indole alkaloids. First isolated from the seeds but chiefly from the pulp of the fruit of *Strychnos nux-vomica* L., *Loganiaceae:* Dunstan, Short, *Pharm. J.* **14,** 1025 (1883); Merz, Krebs, *Arch. Pharm.* **275,** 217 (1937); Merz, Lehmann, *ibid.* **290,** 543 (1957). Structure: Sheth *et al., Tetrahedron Letters* **1961,** 394; Büchi, Manning, *Tetrahedron* **18,** 1049 (1962). Crystal structure: Lentz, Rossmann, *Chem. Commun.* **1969,** 1269; P. G. Jones *et al., Acta Crystallogr.* **B36,** 481 (1980). Abs config: Inouye *et al., Tetrahedron* **26,** 3905 (1970). Total synthesis: Büchi *et al., J. Am. Chem. Soc.* **92,** 2165 (1970); Partridge *et al., ibid.* **95,** 532 (1973); Büchi *et al., ibid.* 540; B.-W. Au-Yeung, I. Fleming, *Chem. Commun.* **1977,** 81; I. Fleming, B.-W. Au-Yeung, *Tetrahedron* **37,** Suppl. 9, 13 (1981); K. Hiroi *et al., Chem. Letters* **1981,** 559. Biosynthetic studies: Battersby, "Biosynthesis II—Terpenoid Indole Alkaloids", in *The Alkaloids* **vol. 1,** The Chemical Society (Burlington House, London, 1971) pp 31-47.



This is Exhibit 8 referred to in the Declaration of Michael M. Lipp, sworn this 9th day of February, 2005.

A Notary Public

CINDY A. SRAGG
Notary Public
My Commission Expires
January 23, 2009

cancer have shown that stimulated T cells can recognise peptides generated from normal cellular components—ie, autoantigens.[2-4,6,11] One picture we get is the potential for autoimmunity directed against cancer. Despite this preliminary impression, cancer immunologists aspire to show that the immune system can respond to mutations and other genetic alterations specifically expressed by tumour cells. The recent report by Kwak et al[13] supports this notion, showing passive transfer of immunity against the tumour-specific idiotype determinant expressed by myeloma.

Can autoantibodies and autoreactive T cells alter the natural history of the cancer? If immunity against these autoantigens can be elicited without undue toxicity, vaccination against these autoantigens may alter cancer progression. Several clinical observations are relevant here. First, in some circumstances the presence of autoantibodies has been correlated with improved prognosis.[14,15] Such an association does not prove causality but it accords with the notion that the presence of autoreactive antibodies can lead to improvement in disease-free and overall survival. Second, patients with cancer who have autoantibodies against glycolipids and other autoantigens have not shown signs or symptoms of autoimmune diseases despite the expression of these antigens by normal tissues. Occasionally, however, individuals with small cell lung cancer develop antoantibodies against the Hu antigen expressed on tumour cells and neurons.[16] Presence of this autoantibody is associated with a more indolent form of the cancer, but also with a sensory polyneuropathy and encephalomyelitis. Third, immunisation with a vaccine incorporating a potential autoantigen, the ganglioside GM2, has shown that most immunised individuals with melanoma can generate autoantibodies specifically against GM2, the vaccine is safe, and the presence of autoantibodies against GM2 is associated with improved survival.[16] This is one of the first experiences of immunisation with a defined molecule, and it is a reasonably good start.

The opportunities for cancer vaccine strategies have grown immensely. Identification of cancer antigens is an essential task, but is not enough for real clinical progress. The choices are daunting. A single type of tumour can present many different antigens. Vaccine possibilities include purified proteins and glycolipids, peptides, viral vectors expressing antigens with or without cytokines, tumour cells genetically altered to express cytokines and co-stimulatory molecules, and a range of immune adjuvants. Clinical and scientific experience will continue to guide research; there may even be a role for intuition.

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Lofexidine and opioid withdrawal

Lofexidine (BritLofex, Britannia Pharmaceuticals) is a structural analogue of the antihypertensive agent clonidine.[1] Lofexidine was originally licensed as an anti-hypertensive in Germany, but was withdrawn because of lack of clinical efficacy. Lofexidine was relaunched in October, 1992, in the UK and is now licensed and promoted as an aid to opioid detoxification. In view of the low side-effect profile of this agent, some practitioners have assumed that it will be useful for outpatient treatment. Moreover, lofexidine's non-opioid nature makes the drug a candidate for use in other settings where the prescription of opioids may be undesirable—eg, in general practice, on medical or surgical wards, and in prisons. Lofexidine's relaunch comes at at time when the Department of Health in Britain is questioning the effectiveness and desirability of more traditional therapeutic options such as methadone maintenance and detoxification. For some years the philosophy of drug agencies has been that of harm reduction, which included the controlled prescribing of replacement drugs. The government Green Paper *Tackling Drugs Together*[2] and a press release by ex-Secretary of State for Health Brian Mawhinney[3] explicitly make withdrawal and abstinence the primary aim of treatment agencies, harm reduction being relegated into second place. This is not only a U-turn in public policy[4] but also ignores decades of experience in the treatment of drug misusers. Moreover, a climate in which detoxification and abstinence are given pride of place will encourage the development of novel and possible costly detoxification strategies at the expense

of attention to the wider problems of drug misusers.

Against this background several other treatment options are being considered. Methadone maintenance in general practice might prove more cost effective than treatment via specialist agencies; ultrarapid detoxification under general anaesthesia has been suggested; and maintenance of abstinence by administration of opioid antagonists such as naltrexone[1] is advocated by some specialists. Despite the undeniable appeal of being able to detoxify opioid misusers quickly and cheaply, the many years of field experience should not be ignored. As drug misusers themselves say, "it's not getting off that's difficult, it's staying off". Detoxification is only the first step on the road to abstinence and rehabilitation; relapse into opioid misuse often occurs months or even years after successful detoxification.

How does lofexidine work and where might it fit into this complex clinical picture? By comparison with clonidine, lofexidine is expensive.[1] Lofexidine has limited effects on blood pressure but retains potent in-vitro noradrenergic antagonist activity. Since many of the symptoms of the opioid withdrawal syndrome are thought to be mediated via noradrenergic pathways,[5] administration of lofexidine should reduce the features of noradrenergic storm[6] such as watery eyes, runny nose, sweating, diarrhoea, chills, and gooseflesh. Clonidine itself has been found to ameliorate these symptoms,[7-9] although the risk of significant hypotension renders it unsuitable for use outside hospital. Other symptoms of opioid withdrawal—notably, bone and muscle pain, insomnia, and, most importantly, craving for the euphoriant effect of opioids—are not relieved by either drug.

Although clonidine has been extensively tested[5] in opioid detoxification, only three trials[10-12] of lofexidine have been reported. There were methodological objections to all of these trials:[5] none were double-blind trials, none were large, and the experimental data necessary to evaluate the results fully were not given in some cases. In general, the studies showed that lofexidine adequately suppressed many although not all of the withdrawal symptoms. As expected, the main reason why detoxification failed in some patients was opioid craving, so it is unlikely that treatment with lofexidine would succeed where clonidine has failed.

All three trials of lofexidine were in methadone users who had been stabilised on relatively low doses, not in heroin users or the poly-drug users typically seen in community settings. A final consideration is that, although lofexidine is not an opioid drug, it may acquire an abuse potential itself. Diverted supplies of lofexidine might allow heroin or illicit methadone users to weather periods of reduced street availability rather than seek contact with services. Experience with drugs such as buprenorphine shows that drug users may resort to using prescribed medication in novel and often undesirable ways—eg, by injecting preparations intended for oral use.

Even if lofexidine proves to be an effective and humane method of opioid withdrawal, that is only one element of addiction management. If opioid misusers are sufficiently well motivated, withdrawal can be achieved with existing medication or even without it. No matter how effective lofexidine is at suppressing withdrawal symptoms it will not provide the motivation to withdraw from opioids and to remain abstinent. Detoxification, by whatever method, is a necessary first step in rehabilitation, but simple detoxification is not enough to help former users to become permanently abstinent. Meanwhile, patients chosen for detoxification with lofexidine will need to be carefully selected by use of the criteria adopted for conventional withdrawal programmes. Such patients should be stable, willing to be seen regularly, willing to try life without the euphoriant effect of opioid drugs, and wise to the hazards of relapse.

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Ever older

See page 1409

"Population aging will be one of the most important social phenomena of the next half century." The conclusion of a panel for the US National Research Council[1] reflects the fact that the number of citizens world wide aged 65 years and older will increase from 328 million in 1990 to 475 million by the year 2005, and to 822 million, or nearly 10% of the living, one generation after that. Policymakers and planners are trying to take stock. So too must doctors; elder care has its own nuances and subtle considerations. We hope our *Geriatrics Septet* will illuminate some.

We owe our contributors much—for prevailing over foreshortened deadlines, faxes passing through uncountable time zones, and editorial requests always to make things shorter, shorter. Above all, we thank them for their excellent work. Our main advisers, Dr Michael Gordon and Dr Paula Rochon of the Baycrest Centre for Geriatric Care and the University of Toronto, Canada, were expert and attentive throughout.

David H Frankel
The Lancet, Santa Barbara, USA

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